NEURAL MECHANISMS IN ABOMASAL MOTILITY.

By

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To my Mother.

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The rest of the work is mine

FOREWORD

´For that which befalleth the sons of men befalleth beasts; even one thing befalleth them; as one dieth so dieth the other; yea, they have all one breath; so that man hath no preeminence above a beast: for all is vanity.

All go onto one place; all are of the dust, and all turn to dust again.

Who knoweth the spirit of man that goeth upward, and the spirit of beast that goeth downward to the earth?

Wherefore I perceive that there is nothing better, than that a man should rejoice in his own works; for that is his portion: for who shall bring him to see what shall be after him?´

Ecclesiastes 3:19-22.

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SUMMARY.

1. The motor profile of the exteriorized abomasal body and antrum of chloralose-anaesthetized adult sheep, recorded using balloon catheters and e.m.g. electrodes, consisted of tonic muscular activity upon which contractile muscular activity could be superimposed. The different motility patterns of the body and antrum suggest that they are separate functional entities.

2. Transection of the exteriorized abomasum into separate body and antral pouches significantly increased antral contraction amplitude and significantly decreased body tone. This suggests that in the intact preparation the body exerts an intrinsic inhibitory drive to antral contraction amplitude, and that either the antrum exerts an intrinsic excitatory drive to body tone or increased antral contraction amplitude results in an extrinsic inhibitory drive to body tone.

3. Repetitive electrical stimulation of the cut peripheral end of the cervical vagus (1-10 Hz, 1-50 V, 1 ms pulse duration for 10 s) caused body relaxation in 5 out of 7 preparations. Higher frequency electrical stimulation (10-50 Hz) caused body contraction. In 4 preparations electrical stimulation (1-50 Hz, 1-50 V, 1 ms pulse duration for 10 s) of the cut peripheral end of the cervical vagus caused antral contraction. In 3 ostensibly identical preparations similar electrical stimulation reduced antral contraction amplitude. Thus evidence was

found for vagal excitatory and inhibitory innervation of the abomasal body and antrum, and for an integrative mechanism at the intramural level.

4. Body pouch inflation increased antral contraction amplitude by a vagally-dependent mechanism, and decreased the discharge rate of 10 out of 33 efferent units isolated from abdominal branches of the vagus within 2 cm of the antrum. If the decrease in unitary discharge is instrumental in increasing antral contraction amplitude it is likely that the units have inhibitory function.

5. The slight body pouch relaxation produced by body pouch inflation was not altered by extrinsic denervation. Inflation of an intra-reticular balloon produced similar effects on the abomasal body motor profile as inflation to the same volume of an intra-abdominal balloon placed beside the reticulum. Thus no evidence was found for a functional reflex corresponding to receptive relaxation found in the monogastric animal.

6. The response of 33 efferent units, dissected from the abdominal continuations of the dorsal and ventral vagi within 2 cm of the antrum, to inflation of the abomasal body pouch, and to systemic injection of 100 ug of adrenaline was investigated. All units responding to body pouch inflation also responded to adrenaline injection. Unitary discharge was analysed for temporal relationships with antral e.m.g. activity and the e.c.g. using a post stimulus time histogram technique. Units showing discharge

association with the antral e.m.g. also showed discharge association with the e.c.g. Thus in the sheep neither discharge response to discrete stimulation of specific receptor populations nor the presence of system-related rhythms in efferent discharge is necessarily indicative of efferent destination or function.

GENERAL INTRODUCTION.

This work concerns an investigation into aspects of the motor control of the abomasum of the chloralose-anaesthetized sheep. With the advent of the era of neurotransmitter multiplicity and the realization of the integrative power of the intrinsic plexi, gastroenterology is becoming an ever more complex field. Because of the paucity of information relating to the abomasal motor profile and the neural mechanisms affecting the abomasal motor profile the implications of these advances to the physiology of the abomasum cannot yet be judged.

Reports pertaining to the sensory and motor electrophysiology of the ovine forestomach (Iggo, 1955; Iggo and Leek, 1967a, 1967b, Harding and Leek 1971, 1972b, 1973) have been published. Characteristics of the afferent innervation of the abomasum and duodenum of the sheep have been described (Harding and Leek, 1972a, 1972b, 1973; Cottrell and Iggo, 1984a, 1984b; Cottrell, D.F. and Reynolds, G.W., personal communication). As yet discharge characteristics of the efferent fibres supplying the abomasum (or indeed the activity of efferent fibres proved to be supplying the monogastric stomach) have not been described. As the abomasum bridges the gap between the forestomach and the duodenum, a better understanding of the neural mechanisms controlling abomasal motility may lead to a better understanding of the neural mechanisms co-ordinating ruminant digestive processes.

CHAPTER ONE

REVIEW OF THE LITERATURE.

NEURAL MECHANISMS IN STOMACH AND ABOMASAL MOTILITY.

1.ABOMASAL ANATOMY.

The ruminant abomasum has the same embryological derivation as the forestomach (Lewis, 1915; Warner, 1958) yet is closer in structure and function to the monogastric stomach. By analogy with the monogastric stomach it is divided into a body, fundus and antrum. The boundary between fundus and body is imprecise in the ruminant; Dyce, Sack and Wensing (1987) suggest that ruminants do not have a true fundus. The abomasal wall is composed of an outer longitudinal muscle layer and an inner circular muscle layer; the longitudinal coat is confined to the curvatures of the body but forms a thicker enclosing layer at the antrum (Dyce, Sack and Wensing, 1987). The mucosa is lined by glandular epithelium arranged in folds. The body mucosa has proper gastric glands, the antrum pyloric glands; a slim area of cardiac glands is located at the omaso-abomasal junction (Nickel, Schummer and Seirferle, 1973).

The abomasum lies ventrally against the right flank. Medially it faces the ventral sac and atrium of the rumen; the reticulum lies cranially and the ventral omasum dorso-cranially. Muscle bundles bind the craniomedial abomasum to the ventral sac, atrium and omasum. The antrum curves

dorsally to meet the duodenum at the duodenal bulb (fig. 1).

2. ABOMASAL MOTILITY.

Pressure and radiographic recordings.

Abomasal pressure and radiographic recordings show body and antrum to have different patterns of motility; the body is quiescent or shows 'occasional' slow waves of contraction whereas the antrum contracts strongly with 'occasional' periods of quiescence (Czepa and Stigler, 1929; Kryzwanek and Quast, 1937; Phillipson, 1939; Stevens, Sellers and Spurrell, 1960; Ohga, Ota and Nakazoto, 1965; Bell, 1978). However, little attempt has been made to quantify the pressure events within the abomasum with respect to tonic muscular activity or frequency or amplitude of contraction.

Electromyographic recordings - abomasal body

Ruckebusch (1970) in conscious adult sheep, found no e.m.g. activity in the resting body, and irregular e.m.g. activity when the sheep was given or shown food. Bell and Grivel (1975) describe grouped discharges at frequencies of 10-40 min^{-1} in the abomasal body of conscious preruminant calves. Bell (1978) recorded e.m.g. activity from both abomasal body and antrum in conscious preruminant calves, but did not describe background activity; his traces do show grouped discharges recorded from the abomasal body. Recently the e.m.g. activity of the abomasal body and antrum of the conscious (Reid and Titchen, 1988) and

anaesthetized (Reid, Schulkes and Titchen, 1988a) milk-fed lamb have been found to differ. In conscious fasted lambs the e.m.g. of the body consists of individual spikes or salvos of spikes of up to 10 s in duration at a frequency of 6-12 min^{-1}; spiking activity occurs in periods of 1-20 min separated by periods of quiescence of 0.5-10.0 min. Feeding increases salvo voltage, duration and period. In the anaesthetized lamb, body e.m.g. activity consists of salvos of spikes 2-10 s in duration at a frequency of 4-15 min^{-1}; there were no periods of quiescence. Pressure correlates of these e.m.g. events were not recorded.

Electromyographic recordings - abomasal antrum.

The antral e.m.g. activity has received more detailed attention than the body. In conscious sheep, Ruckebusch (1970) describes a slow wave with bursts of spikes at a frequency of approximately 7 min^{-1} and a propagation velocity of 5 cm s^{-1}. This contrasted with a later report (Ruckebusch and Bueno, 1977) where antral propagation velocity was calculated as 0.7 +/- 0.2 cm s^{-1} and groups of 4-5 slow waves with spiking activity were separated by groups of 2-3 slow waves without spiking activity. Ten min periods of antral e.m.g. quiescence coincided with bursts of regular spiking activity on the duodenum at intervals of 60-90 min (Ruckebusch and Bueno, 1977). Reid and Titchen (1988) recording antral e.m.g. activity in the milk-fed lamb found that salvo frequency was ca. 6 min^{-1} occurring synchronously with those of the body. Feeding increased the voltage and duration of the e.m.g. salvos (Reid and

Titchen, 1988). Reid, Schulkes and Titchen (1988a) found that antral e.m.g. activity of the preruminant anaesthetized lamb differed in that the salvos of spiking activity were more irregular (3-5 min^{-1}), and spiking activity was continuous. Pressure correlates of these e.m.g. events were not recorded.

3. ABOMASAL EXTRINSIC INNERVATION.

The abomasum has an intrinsic and extrinsic innervation. Extrinsic innervation is supplied by the vagal and splanchnic nerves. Cottrell and Greenhorn (1987) describe cell bodies from afferent fibres supplying the gastro-duodenal junction in the left and right nodose ganglia, and gastro-duodenal junction vagal efferent cell bodies in the dorsal motor nucleus of the vagus in the sheep. Sympathetic postganglionic fibres from the stellate ganglion anastamose with the left and right vagal trunks in the thorax. There is a variable number of **intrathoracic anastomoses** of the vagal trunks; the final **anastomoses** rotates the trunks to form dorsal and ventral branches on the oesophagus as it enters the abdomen (Habel, 1956). Sympathetic postganglionic fibres from the coeliac ganglion join the dorsal and ventral trunks at this level (Habel, 1956). The abomasum is innervated by the 'abdominal continuation of the ventral vagus' (Duncan, 1953; Habel, 1956), the 'abdominal continuation of the dorsal vagus' (Duncan, 1953; Habel, 1956) and the 'pyloric branch of the hepato-duodenal nerve' (Duncan, 1953; Habel, 1956; Cottrell and Greenhorn, 1987). As the so-called 'abdominal continuations of the dorsal and ventral vagus' contain both

splanchnic and vagal fibres they are both clumsily and badly named. Cell bodies of ovine splanchnic afferents have been identified in the dorsal root ganglia T6-L3, though this work concentrated on the gastro-duodenal junction (Cottrell and Greenhorn, 1987). No study has been made of the location of sympathetic preganglionic cell bodies supplying the ovine abomasum . In the monogastric stomach sympathetic fibres originate in the thoracic ventral horn from T6-T9; stomach sympathetic postganglionic cell bodies occur in the coeliac ganglion (Youmans, 1968).

4. VAGAL INFLUENCE ON STOMACH AND ABOMASAL MOTILITY.

Vagal excitatory innervation of the stomach and abomasum.

Electrical stimulation of the peripheral end of the cut cervical vagus may elicit either contraction or relaxation of the monogastric stomach (Langley, 1898; Paton and Vane, 1963; Martinson and Muren, 1963; Andrews and Scratcherd, 1980). The effect of excitatory fibre stimulation is reduced by hexamethonium at the ganglionic level and eliminated by atropine at the neuromuscular junction (Bulbring and Gershon, 1967; Beani, Bianchi and Crema, 1971; Andrews and Scratcherd, 1980). Residual excitatory effects of electrical stimulation of the peripheral end of the cut vagus after administration of hexamethonium may be due to muscarinic (m)-cholinergic transmission at the ganglionic level (Changeaux, 1987), or to the effect of antidromic stimulation of afferents, or to the release of more than

one neurotransmitter by the preganglionic fibres. The effect of selective antidromic stimulation of stomach afferents is an unresearched field, but the presence in vagal afferents of peptides with putative neurotransmitter function is well documented (Hokfelt, Elde, Johansson, Luft, Nilsson and Arimura, 1976; Dockray, Gregory, Tracey and Wen-Yu Zhu, 1981; Gilbert, Emson, Fahrenbrug, Lee, Penman and Wass, 1980; Rehfeld,1983). Bulbring and Gershon (1967) and Bingham (1987) suggest that 5-HT has a neurotransmitter function on the vagal postganglionic fibres of the stomach of mice and guinea pigs, and ferrets respectively.

Andrews and Lawes (1985b) propose, on the basis of the effect of supramaximal electrical stimulation of the cut peripheral end of the cervical vagi, that the left and right cervical vagi make equal contributions to the motor innervation of the ferret stomach. Electrical stimulation of the dorsal abdominal vagus elicits larger amplitude contractions of the stomach than similar stimulation of the ventral abdominal vagus (Andrews, Lawes and Bower, 1980). The same authors, on the basis of the magnitude of stomach contractions elicited by electrical stimulation of nerves propose total functional overlap of the right and left, and dorsal and ventral vagal trunks as concerns stomach motility in the ferret (Andrews, Lawes and Bower, 1980). Ascribing equality of 'functional' overlap on the basis of the quantitative effects of electrical stimulation of nerves is a questionable technique.

In monogastric animals as the frequency of repetitive

electrical stimulation of the peripheral end of the cut vagus increases, so too does the amplitude of elicited stomach contraction to a point where maximum transmitter release occurs and the frequency response curve levels out (Paton and Vane, 1963; Martinson, 1964; Beani, Bianchi and Crema, 1971; Burns and Reinke, 1971; Andrews, Scratcherd and Wynne, 1976; Andrews and Scratcherd, 1980; Andrews, Lawes and Bower, 1980). The frequency at which maximum elicited stomach contraction amplitude, and therefore greatest excitatory transmitter release is achieved appears to differ between species. Maximum contraction amplitude in cats (Martinson, 1964) and ferrets (Andrews and Scratcherd, 1980; Andrews, Lawes and Bower, 1980) is elicited by stimulation of the peripheral cut end of the vagus at frequencies of 5-10 Hz. Stimulation at 20 Hz produces maximum contraction amplitude in guinea pigs (Paton and Vane, 1963; Beani, Bianchi and Crema, 1971). Burns and Reinke (1971) found maximum contraction amplitudes occurred in rabbits at stimulation frequencies of 16-32 Hz.

There are no reports that different frequencies of electrical stimulation of peripheral nerves affect the frequency of contraction of the stomach.

The duration of electrical stimulation of the peripheral end of the cut vagus affects the pattern of response of the monogastric stomach. Stimulus durations of 10 s or less evoke single contractions in both the body and the antrum (Brooks and Carr, 1975; Andrews and Scratcherd, 1980). Longer periods of stimulation have different effects on

body and antrum. The body reacts with a large increase in tone with smaller contractions of varying amplitude superimposed (Brooks and Carr, 1975; Andrews and Scratcherd, 1980). Brooks and Carr (1975), working with cats, only maintained their stimulus for 30 s. Andrews and Scratcherd (1980) working with ferrets found that the electrically evoked increase in body tone and contraction amplitude returned to baseline values over a period of 2-4 min although the stimulus was maintained. Increases in stomach tone and contraction amplitude evoked by 'natural' stimuli such as cytoglucopenia or stomach distension do not show this diminution of response if the stimulus is maintained (Andrews and Scratcherd, 1980) highlighting the dangers of physiological investigation using non-physiological stimuli.

Antral response to longer periods of electrical stimulation of the peripheral end of the cut vagus is similar in cats (Brooks and Carr, 1975) and ferrets (Andrews and Scratcherd, 1980); large amplitude contractions occurred with minimal tone change. The amplitude of antral contractions do not decrease with prolonged electrical stimulation of the cut peripheral end of the cervical vagus (Andrews and Scratcherd, 1980).

The only reference that has been found to the effect of electrical stimulation of the peripheral end of the vagus on the abomasal motor profile is that of Reid, Shulkes and Titchen (1988a). They describe the effect of electrical stimulation (10 Hz, 5 ms, 10V) of the peripheral end of the cut cervical vagus on the e.m.g. of the body, antrum

and pylorus of milk-fed lambs as resulting in 'an initial increase in the voltage and frequency of the e.m.g. activity at all three sites with, however, reduced activity towards the end of or after stimulation for 180 s.' Abomasal pressures were not recorded. It is difficult to compare this account with the described effects of electrical stimulation of the peripheral end of the cut vagus on the stomach of monogastric animals.

Although the monogastric stomach and ruminant abomasum, unlike the ruminant forestomach (Duncan, 1953; Gregory, 1982, 1984) do not rely on vagal drive (Duncan, 1953; Aune, 1969; Koster and Madsen, 1970; Bell, 1978) their inherent motility is strongly influenced by both excitatory and inhibitory reflexes with efferent limbs in the vagus. Excitatory drive to the stomach can be elicited by mechanical stimulation of the stomach and of other regions. Stomach distension has been implicated in the regulation of stomach emptying in man (Hunt and McDonald, 1954; Erskine and Hunt, 1981; Bateman, 1982), dog (Strunz and Grossman, 1978) and ferret (Andrews, Grundy and Scratcherd, 1980a; Andrews and Scratcherd, 1980). Abomasal distension has been implicated in the regulation of abomasal emptying in the pre-ruminant calf (Bell and Watson, 1976; Bell, Holbrooke and Titchen, 1977). Andrews and Scratcherd (1980) have shown that stomach distension increases antral contraction amplitude in a vagally dependent manner. Andrews, Grundy and Scratcherd (1980a) suggested that body distension increases antral contraction amplitude via a vagal reflex. This requires confirmation because, although

the body and antrum were surgically transected in their preparation, they did not exclude the possibility that body inflation increases antral contraction amplitude indirectly by causing an increase in antral pressure and thus eliciting an antro-antral excitatory reflex. In their favour is the fact that although an antro-antral excitatory reflex has been described in the rabbit (Deloof and Rousseau, 1985; Deloof, Bennis and Rousseau 1987) and ferret (Grundy, Hutson and Scratcherd, 1986) it is mediated by an intramural and not by a vago-vagal pathway.

There is evidence in both monogastrics and ruminants to suggest that the stomach and abomasum have distension-sensitive enteroreceptors. The existence of abomasal distension-sensitive receptors is implied by the work of Phillipson (1939) and Titchen (1958). Distension-sensitive 'in-series' (Iggo, 1955) mechanoreceptors in both the monogastric stomach (Paintal, 1953, 1954; Iggo, 1955; Clarke and Davison, 1974; Andrews, Grundy and Scratcherd, 1979, 1980b) and ruminant abomasum (Harding and Leek, 1972a) have been identified by single unit recordings at the cervical level. Unitary activity of the dorsal vagal nucleus of sheep (Harding and Leek, 1973) and lateral hypothalamus of cats (Jeanningros, 1984) is modulated by gastric distension. Cervical vagus efferent discharge is modulated by stomach distension in the rat (Davison and Grundy, 1976, 1977, 1978a, 1978b) and ferret (Grundy, Salih and Scratcherd, 1981; Blackshaw, Grundy and Scratcherd, 1987). It is clear that the stomach and abomasum are equipped with the means to influence their own motility via

vago-vagal reflexes. Cytoglucopenia induced by administration of 2 deoxy-D-glucose promotes stomach motility via vagal efferents in rats (Kadekaro, Timo-Iara and Vicentini, 1977); also in the rat pinching of the hindpaw facilitates gastric motility by a cutaneo-gastric somato-vagal reflex (Kametani, Sato, Sato and Simpson, 1979).

There is evidence that vagal preganglionic excitatory fibres may play a permissive, indirect role in the mediation of antro-antral excitatory reflexes. Antral distension causes increased amplitude of antral contraction in the rabbit (Deloof and Rousseau, 1985) and ferret (Grundy, Hutson and Scratcherd, 1986). Both groups found that vagotomy greatly reduced but did not eliminate this response. Grundy et al (1986) found that either close arterial infusion of acetylcholine or low frequency stimulation of the peripheral transected vagus re-established the original magnitude of response and suggested that the response is mediated via the intramural plexus, and that the preganglionic influence is permissive.

Vagal inhibitory innervation of the monogastric stomach and ruminant abomasum.

Most reports agree that in monogastric animals vagotomy produces an increase in stomach pressure and a decrease in stomach reservoir capacity (Aune, 1969; Koster and Madsen, 1970; Carter, Whitefield, and McLeod, 1972; Andrews, Grundy and Lawes, 1980; Andrews and Lawes 1982, 1984). This suggests the presence of a tonic vagal inhibitory influence

and a vagally mediated ability to increase reservoir capacity. Conversly, Aspiroz and Malegelada (1987) have by cooling the vagus induced a decrease in stomach tone in the fasted dog, suggesting that the tonic effect of vagal input may vary.

Further evidence for the existence of a vagal inhibitory supply to the stomach comes from studies of reflexes. In the cat distension of the oesophagus (Abrahamsson and Jansson, 1969; Jansson, 1969), entire stomach (Abrahamson, 1973a), antrum (Abrahamsson, 1973a, 1973b) and stimulation of pharyngeal mechanoreceptors (Abrahamsson and Jansson, 1969; Jansson, 1969) elicits stomach relaxation by a vagally-dependent mechanism. Stomach distension elicits a vago-vagal gastro-gastric relaxatory reflex in the ferret (Andrews, Grundy and Lawes, 1980; Andrews and Scratcherd, 1980; Andrews and Lawes, 1982). Stomach relaxation can be also be elicited by electrical stimulation of the cut central end of the cervical vagus in the cat (Harper, Kidd and Scratcherd, 1959; Jansson, 1969; Abrahamsson, 1973a) if the contralateral vagus is intact. Antral e.m.g. activity may be reduced by electrical stimulation at 10-15 Hz of the central cut end of the cervical vagus of the rabbit (Deloof, Bennis and Rousseau, 1987) if the contralateral vagus and spinal cord above T6 is intact. These relaxatory events persist in the presence of m-cholinergic and adrenergic blocking agents and are therefore not due to a reflexly-induced decrease in the activity of the vagal postganglionic cholinergic excitatory fibres or a reflexly-induced increase in the activity of adrenergic inhibitory

fibres. The authors propose that the stomach relaxation is elicited by a reflexly-induced increase in the activity of vagal non-adrenergic, non-cholinergic inhibitory fibres. This is only tenable if it is assumed that all postganglionic vagal excitatory action is blocked by atropine. There is evidence for non-cholinergic excitatory neurones in the intestine of the guinea pig (Bywater, Holman and Taylor, 1981; Smith, Furness, Costa, and Bornstein, 1988) and ferret (Collman, Grundy and Scratcherd, 1983, 1984). If similar non-cholinergic excitatory neurones exist in the stomach, electrical stimulation of the central cut end of the vagus could produce atropine-resistant stomach relaxation by reflexly reducing the activity in vagal preganglionic neurones.

Further evidence for the existence of a vagal non-adrenergic, non-cholinergic innervation of the stomach comes from the results of electrical stimulation of the cut peripheral end of the vagus. Harper, Kidd and Scratcherd (1959) found that stimulation of the cut peripheral end of the vagus in cats causes contractions superimposed on a drop in stomach tone. They ascribed the tone drop to the action of inhibitory fibres. Other workers have described a contractile response by the stomach to electrical stimulation of the peripheral end of the cut vagus followed by an immediate and slow-recovering fall in stomach pressure when electrical stimulation stopped. (Andrews and Scratcherd, 1980; Andrews and Lawes, 1985b). This may be due to release of acetylcholine and one or more relaxatory neurotransmitters with longer half life than acetylcholine.

The full relaxatory effect of electrical stimulation of the peripheral end of the vagus is best seen after nullification of the vagal cholinergic excitatory input to the stomach by atropine as described in the cat (Martinson and Muren, 1963; Martinson, 1965c; Jansson and Martinson, 1965), guinea pig (Campbell, 1966; Bulbring and Gershon, 1967), rat (Aihara, Nakamura, Sato and Simpson, 1978; Andrews and Grundy, 1981) and ferret (Andrews and Scratcherd, 1980; Andrews and Lawes, 1985a, 1985b).

The only evidence for a vagal non-adrenergic, non-cholinergic innervation of the abomasum is that of Reid, Schulkes and Titchen (1988a) who showed that the e.m.g. activity of the abomasal body, antrum and pylorus may be inhibited by electrical stimulation of the peripheral end of the cut vagus of atropinized lambs. No pressure correlate was taken.

The possible effects of antidromic impulses produced by electrical stimulation of afferent fibres should not be forgotten in this type of work, especially if inferences are made as to the nature of putative inhibitory neurotransmitters.

Vagal preganglionic inhibitory fibres, like their excitatory counterparts, are blocked by n-cholinergic blocking agents (Martinson, 1965a; Jansson, 1969; Bulbring and Gershon, 1967; Beani, Bianchi and Crema, 1971; Andrews and Lawes, 1982, 1985a). Bulbring and Gershon (1967) and Bingham (1987) suggest that 5-HT may have transmitter function at the vagal preganglionic layer, but Beani et al

(1971) found no evidence for this.

The nature of the vagal inhibitory postganglionic neurotransmitter is disputed. Paton and Vane's (1963) suggestion that the vagal inhibitory neurotransmitter is noradrenaline is refuted by the results of Campbell (1966), Abrahamsson and Jansson (1969), Beani, Bianchi and Crema (1971), Andrews and Grundy (1981), Andrews and Lawes (1985a) and Deloof, Bennis and Rousseau (1987) who found that vagally-induced stomach relaxation is not blocked by adrenergic antagonists. Burnstock, Campbell, Satchell and Smythe (1970) suggest that the vagal inhibitory neurotransmitter is purinergic, possibly ATP. Andrews and Lawes (1985a) found that close arterial ATP and ATP analogue infusion produces small contractions in the ferret stomach; they also discounted bradykinin, CCK-8, substance-P, pentagastrin and bombesin for the same reason. Close arterial infusion of neurotensin and vasoactive intestinal polypeptide (VIP) causes stomach relaxation; the time course of VIP-induced relaxation most closely mimicked vagally-induced relaxation in their preparation (Andrews and Lawes, 1985a). Reid, Shulkes and Titchen (1988a) working with atropinized lambs ascribed inhibitory neurotransmitter function to VIP as electrical stimulation of the peripheral end of the cut vagus increased VIP concentration in gastric venous effluent.

Deloof, Croix and Tramu (1988) propose VIP as a stomach inhibitory neurotransmitter in the rabbit on the basis of: a) demonstrating that close intra-arterial

injection of VIP inhibited antral electrical activity; b) identifying VIP in antral nerve terminals; and, c) equating the inhibition of antral motility produced by electrical stimulation of the central cut end of the vagus at the antral level with increase in the titre of VIP in portal blood. They were, however, unable to measure the effect of electrical stimulation of the vagus on the rate of portal blood flow (Deloof, Croix and Tramu, 1988).

The electrical threshold required to elicit cervical vagally-induced stomach relaxation in the cat is higher than that required to elicit contraction (Martinson and Muren, 1963; Martinson, 1964, 1965b, 1965c; Jansson and Martinson, 1965). Andrews and Lawes (1985a) could find no such difference between excitatory and inhibitory stomach efferents in the ferret. Care must be taken when interpreting electrical threshold differences between excitatory and inhibitory stomach innervation as Andrews and Lawes (1985b) have shown that presiding stomach tone influences the response of the stomach to extrinsic neural stimuli. Electrical stimulation of the cut peripheral end of the cervical vagus produces a smaller amplitude of contraction in a stomach containing 60 ml of fluid than in the same stomach containing 20 ml of fluid (Andrews and Lawes, 1985b). This may explain the difference in reports of the effect of vagotomy on stomach tone (Carter, Whitefield and McLeod, 1972; Aune, 1969; Korster and Madsen, 1970; Andrews and Lawes, 1982, 1984; Aspiroz and Malagelada, 1987).

Activation of the vagal inhibitory fibres affects the

stomach body and antrum in different ways. Andrews and Scratcherd (1980) transected the stomach of an atropinized ferret into separate body and antral pouches and noted the results of electrical stimulation of the peripheral end of the cut cervical vagus. Body pressure falls, returning slowly to the resting level on cessation of electrical stimulation. Antral spontaneous contractions are inhibited by vagal stimulation, but no significant change in baseline pressure occurs (Andrews and Scratcherd, 1980).

5. VAGAL EFFERENT ACTIVITY IN RELATION TO THE STOMACH AND ABOMASUM.

Because of the preponderance of efferent units with pulmonary and cardiovascular discharge rhythms in the cervical vagus multiunit recordings provide little information about putative gastric efferents; single unit studies are necessary. There are technical disadvantages in studying putative stomach efferent nerves by recording unitary activity at the cervical or central levels. Firstly, efferent function and destination remain a matter of conjecture. It is not tenable to judge efferent destination solely on response to organ manipulation. For example Davison and Grundy (1977) recording from the cervical vagus of rats identified four types of efferent unit that responded to stomach distension; a proportion of these units also responded to duodenal and colonic distension. Similarily the activity of a single unit, ascribed reticulum function in the sheep by Iggo and Leek (1967b), was modulated by acidification of the abomasum.

Thus central convergence of afferents from different sources onto interneurones or efferents means that extreme care must be taken in ascribing efferent function or destination on the basis of efferent discharge response to stimulation of sensory receptor populations. Secondly, when trying to isolate stomach-associated units at the cervical level it has been usual for units with overt cardiovascular or respiratory rhythms to be disregarded. There is no theoretical justification for this; the degree of convergence of a) afferents from disparate sources and b) centrally-located oscillation circuits onto central systems controlling efferent output may lead to efferent discharge rhythms unrelated to the function of the organ supplied by that efferent (Adrian, Bronk and Phillips, 1932; Cohen and Gootman, 1970; Gregor, Janig and Wiprich, 1977; Kolloi and Koizumi, 1980; Barman, 1984; Bahr, Bartel, Blumberg and Janig, 1986b).

Four types of vagal efferent response to monogastric stomach inflation have been described by Davison and Grundy (1976, 1977, 1978a, 1978b) recording at the cervical level in the rat. The activity of type I units increases with gastric distension, that of type II decreases with gastric distension. Type III unit discharge increases with moderate distension, but decreases at higher distension levels; type IV unit discharge decreases with moderate distension but increases with higher levels of distension. Type I units were further subdivided into those with tonic activity (type Ia) and those active only during contractions of the stomach (type Ic). In the ferret units

corresponding in behavior to types I, II and III (in the ratio 55:44:1.8) (Grundy, Salih and Scratcherd, 1981) have been described although, in the same species, Andrews, Salih and Scratcherd (1978) and Blackshaw, Grundy and Scratcherd (1987) have identified unitary behavior corresponding to types I and II only.

No electrophysiological study has been made of the efferent supply to the ruminant abomasum, but Iggo and Leek (1967a, 1967b) recording at the cervical level identified seven efferent types associated with the motility patterns of the ovine reticulum and rumen. Identification and characterization of unit type was made on the basis of temporal relation of the discharge pattern with muscular activity of regions of the reticulum and rumen, and on association of the discharge response of the units to manipulation of reticulorumen motility. Types I, II and III innervate the reticulum, type IV the rumen and types V, VI and VII 'gastric structures not yet identified'.

Central recording of unitary activity allows tracing of the unit to its target organ as axon continuity is maintained. Andrews, Duthie, Fussey and Mellersh (1978) identified units in the dorsal vagal motor nucleus of the dog with axons projecting to the stomach wall. The spontaneous discharge of these units was irregular; interval histograms showed some random distribution of interspike intervals, some a clear unimodal distribution. The event to which this unimodal distribution was related was not identified. Harding and Leek (1971, 1972b, 1973), in the sheep, identified three populations of forestomach-

associated medullary neurones with 'afferent-like', interneurone and motorneurone characteristics respectively. No attempt was made to look for the projection of the motoneurones.

Efferent destination may be investigated by indirect means. Moilan and Roman (1978) by suturing the cut central end of the left thoracic vagus to the peripheral end of the cut phrenic nerve in dogs were able to correlate the e.m.g. activity of re-innervated diaphragmatic muscle cells with the migrating myoelectric complex of the stomach antrum and thus implicate left vagal innervation of the antrum. The presence of antral rhythms in the discharge of these units may be a result of afferent convergence instead of an implication of efferent function or destination.

6. SPLANCHNIC INFLUENCE ON STOMACH AND ABOMASAL MOTILITY.

Splanchnic inhibitory influence on stomach amd abomasal motility.

Pearcy and Van Liere (1926) observed that severe distension of any portion of the gastrointestinal tract of dogs or cats causes inhibition of the motility of other portions of the gastrointestinal tract with a latency of response faster than would be expected if the initiation of inhibition had a humoral basis. In his review Youmanns (1944) concluded that the vagal nerves played no part in this gastrointestino-gastrointestinal inhibitory reflex, and later proposed that it could be mediated by three pathways; via the splanchnic nerves, via local reflexes

through the prevertebral ganglia, and via axon reflexes (Youmanns, 1968). Recent studies have confirmed the role of splanchnic reflex arcs in inhibition of stomach motility by visceral stimulation (Jansson and Martinson, 1966; Jansson, 1969; Jansson and Lisander, 1969; Abrahamsson, 1973a; Andrews, Grundy and Lawes, 1980; Deloof and Rousseau, 1985; Lisander and Delbro, 1987). Inhibition of stomach motility can also be achieved by cutaneous stimulation via somatosplanchnic reflex arcs (Sato, Sato, Shimata and Torigata, 1975; Kamatani, Sato, Sato and Simpson, 1979; Nosaka, Sato and Shimada, 1980).

Electrical stimulation of splanchnic efferents may cause inhibition of stomach motility via adrenergic neurones (McSwiney and Wadge, 1928; Paton and Vane, 1963; Jansson and Martinson, 1966; Jansson, 1969; Jansson and Lisander, 1969; Aihara, Nakomura, Sato and Simpson, 1978; Andrews and Lawes, 1984, 1985b). By exploiting the difference in threshold to electrical stimulation of B-fibres and C-fibres, Aihara, Nakomura, Sato and Simpson (1978) showed in rats that the splanchnic inhibitory innervation of the stomach consisted of both B-fibres and C-fibres although the B-fibres gave the largest contribution.

The site of action of splanchnic postganglionic inhibitory efferents is disputed. Jansson and Martinson (1966), Jansson (1969) and Jansson and Lisander (1969) report that in cats efferent splanchnic electrical stimulation has little effect on the stomach after atropinization or after vagotomy. The same authors, and Abrahamsson (1973b, 1974) found that the gastro-gastric

inhibitory reflex can only be demonstrated in the presence of vagal tone. They concluded that the splanchnic postganglionic inhibitory efferent fibres to the stomach act mainly by inhibition of vagal cholinergic post-ganglionic neurones (Jansson and Martinson, 1966; Jansson, 1969; Jansson and Lisander, 1969; Abrahamsson, 1973b, 1974). Deloof and Rousseau (1985) found that inflation of the rabbit antrum increased e.m.g. burst frequency, and that deflation prolonged the first post-deflation antral e.m.g. burst interval. Splanchotomy decreased the delay of the first post-deflation interval suggesting removal of an inhibitory splanchnic input. However the effect of splanchotomy was abolished by prior vagotomy, so they also concluded that the site of stomach splanchnic efferent inhibition was at the vagal postganglionic level (Deloof and Rousseau, 1985). Conversely, Andrews and Lawes (1984, 1985b) working with ferrets found that the extent of splanchnically-induced stomach relaxation is dependent on preceding stomach tone and is not dependent on vagal integrity. They concluded that splanchnic inhibitory postganglionic fibres act directly on smooth muscle. It is possible that the reduction of splanchnic inhibitory capacity by atropine or vagotomy reported by Jansson (1969), Jansson et al (1966, 1969), Abrahamsson (1973a, 1974) and Deloof and Rousseau (1985) is an indirect effect mediated by atropine- or vagotomy-induced loss of stomach tone. Furthermore, although splanchnic section in the ferret reduces the stomach relaxatory response to stomach inflation, suggesting the removal of a splanchnic

relaxatory input, this reduction of relaxation is only significant after vagotomy or after atropinization (Andrews, Grundy and Lawes, 1980; Andrews and Lawes, 1984). This suggests that the vagus suppresses splanchnic activity in the intact animal, and, as splanchnic mediated relaxation is not reduced by electrical stimulation of the cut peripheral end of the vagus that this suppression is centrally rather than peripherally mediated (Andrews and Lawes, 1984). This ties in with the results of Sato, Sato, Shimada and Torigata (1975) who found that sympathetic motoneurone activity is increased in vagotomized animals, and also with the results of Lisander and Delbro (1987) who found that hypothalamic stimulation in cats counteracts sympathetically mediated stomach relaxation by a mechanism requiring spinal integrity.

It is therefore possible that peripheral splanchnic inhibition occurs at both the ganglionic and neuromuscular junction levels.

Splanchnic excitatory innervation of the stomach and abomasum.

Electrical stimulation of the splanchnic efferent nerves of monogastric animals at low frequency and voltage levels may induce stomach contractions (McSwiney and Robson, 1931; Semba and Hiraoka, 1957; Jansson and Martinson, 1966). These fibres are cholinergic (McSwiney and Robson, 1931) and have been shown to synapse distal to the prevertebral ganglia (Semba and Hiroaka, 1957).

Local heating of the mucosa of extrinsically denervated

feline stomachs elicits stomach contractions; this response is resistant to nicotinic and adrenergic blockade but is abolished by atropine or prior splanchnic afferent degeneration (Delbro, Lisander and Andersson, 1984). They propose that the contractions are induced by axon reflexes conveyed by splanchnic afferents that, possibly by release of substance P, activate intramural excitatory cholinergic neurons.

7. THE ROLE OF THE SPLANCHNIC INNERVATION OF THE STOMACH AND ABOMASUM.

The role of the splanchnic nerves in the control of stomach motility under 'physiological' conditions is not clear. Duncan (1953) found that splanchotomy did not affect abomasal activity in sheep, but Bloom, Edwards and Hardy (1978) observed that splanchotomy reduced the rate of abomasal emptying in milk-fed calves. Abrahamson (1973b) proposed that gastric relaxation is mediated by both vagal and splanchnic inhibitory fibres in the cat. In ferrets splanchotomy reduces the ability of the stomach to relax to accommodate any increase in volume, but this reduction is only significant after vagotomy (Andrews, Grundy and Lawes, 1980).

8. SPLANCHNIC EFFERENT ACTIVITY IN RELATION TO THE STOMACH AND ABOMASUM.

Because of the relative proximity of the splanchnic nerves to the structures they innervate multifibre recordings might be expected to yield more information

about the characteristics of gastrointestinal innervation than multifibre recordings from the cervical vagus. Many analyses of splanchnic multifibre efferent activity have been made (Adrian, Bronk and Phillips, 1932; Gernandt, Liljestrand and Zotterman, 1946; Koizumi, Seller, Kauffman and McChandler Brooks, 1971; Cohen and Gootman, 1970; Bower, 1975; Nosaka, Sato and Shimada, 1980). The results of each analysis are in general agreement; splanchnic multifibre discharge shows cardiovascular and respiratory rhythms. Perhaps the most detailed analysis was that of Cohen and Gootman (1970) who found three inherent periodicities in spontaneous splanchnic multifibre discharge in cats: a 10 s^{-1} wave synchronized to a greater or lesser extent in a 3:1 ratio with the cardiac cycle: a periodicity with a 1:1 rhythm with the cardiac cycle proposed to arise from baroreceptor input: a centrally arising oscillation in phase with the ventilation rate. This latter could be modulated by vagal pulmonary afferent input.

This preponderance of cardiovascular and respiratory rhythms has been described in sympathetic multifibre recordings from skeletal muscle (Deluis, Hagbarth, Hongell and Wallin, 1972a), thoracic viscera (Seller, 1973; Koizumi, Seller, Kaufman and McChandler Brooks, 1971) and the hypogastric nerve (Adrian, Bronk and Phillips, 1932). No sympathetic multifibre recording has been described with a discharge pattern which correlates with stomach activity. Single unit studies of lumbar splanchnic efferents of cats have identified sympathetic preganglionic

unitary activity that can be classified into 'motility regulating' (MR) or 'visceral vasoconstrictor' (VVC) by their response to mechanical stimulation of the colon and bladder (Bahr, Bartel, Blumberg and Janig, 1986a, 1986c; Bartel, Blumberg and Janig, 1986) and manipulation of blood pressure and blood oxygen tension (Bahr, Bartel, Blumberg and Janig, 1986b). Visceral vasoconstrictor neurones have pronounced cardiac rhythmicity and their activity can be correlated with the cycle of artificial ventilation. Stimulation of arterial baroreceptors by increase in blood pressure causes a decrease in the discharge rate; off-loading of baroreceptors increases discharge rate. Only 14% of VVC neurones were affected by manipulation of colon and bladder. The mean conduction velocities of VVC neurones is 2.8 +/- 2.5 m s^{-1}; mean conduction velocities of the MR neurones is 8.1 +/- 4.7 m s^{-1}. The MR neurones behave reciprocally to bladder and colon distension and are subclassified accordingly. MR1 neurone activity is increased by bladder distension and decreased by colon distension; MR2 neurone activity is decreased by bladder distension and increased by colonic distension.

Floyd, Hick and Morrison (1982) and Janig, Schmidt, Schnitzler and Wesselman (1986) have identified sympathetic postganglionic sympathetic efferents in the cat hypogastric nerves which also respond to manipulation of the bladder and/or colon although their reports differ in detail. The units described by Janig et al (1986) follow the MR1 and MR2 classification defined for sympathetic lumbar preganglionic units by Bahr et al (1986), responding

reciprocally to colon and bladder distension. Floyd et al (1986) found that the discharge of 37.5% of the units studied was affected similarily by bladder distension, contraction and colonic distension; no reciprocally responding units were identified. Floyd et al (1982) found no units with overt cardiovascular or respiratory rhythms, attributing this to having denervated the baroreceptors beforehand. This is surprising as other workers have found cardiovascular and respiratory rhythms in feline hypogastric (Adrian, Bronk and Phillips, 1932; Priess, Kirchner and Polosa, 1975) and thoracic splanchnic (Cohen and Gootman, 1970) multifibre recordings after baroreceptor denervation and attributed this to imposition by the respiratory centres of the central respiratory drive potential on the vasomotor centres.

Splanchnic multifibre efferent discharge can be modulated by cutaneous stimulation via somatosplanchnic reflex arcs in the rat (Kametani, Sato, Sato and Simpson, 1978, 1979; Koizumi, Sato and Terui, 1980; Nosaka, Sato and Shimada, 1980).

9. DISTENSION SENSITIVE RECEPTORS OF THE RETICULUM AND THE REFLEX EFFECTS OF THEIR ACTIVATION.

'In series' tension receptors (i.e. responding to both distension of viscus and contraction of the muscle containing the viscus) occur in the wall of the reticulum (Iggo 1954, 1955; Leek, 1969). Reticular distension has been implicated in reflex arcs influencing reticulo-ruminal

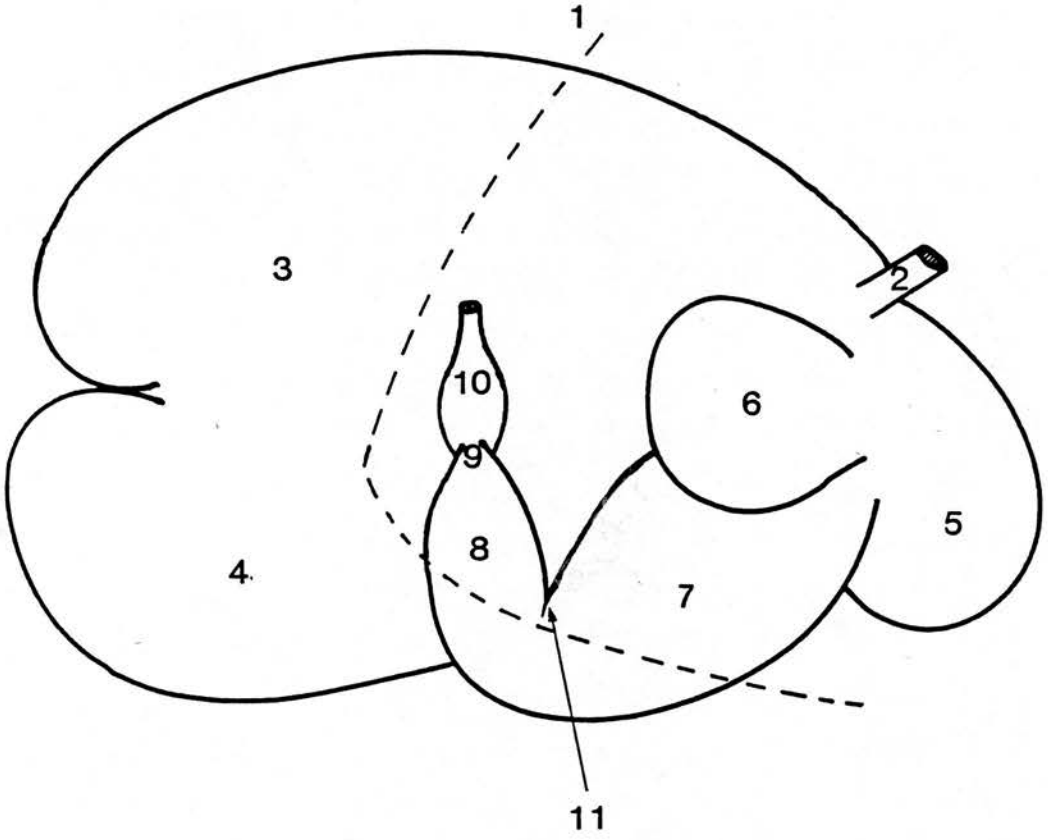
motility (Iggo, 1956; Titchen, 1958; Ash and Kay, 1959; Kay and Phillipson, 1959), salivation (Ash and Kay, 1959) and in monitoring 'rumen fill' (Campling, 1970). Reticular distension has also been shown to modulate the discharge of vagal preganglionic motorneurones (Iggo and Leek, 1967a, 1967b; Harding and Leek, 1971, 1973).

Many people have noted an association between the movement of the reticulum and abomasal motility (Schalk and Amadon, 1928; Czepa and Stigler, 1929; Magee, 1932; Kryzwanek and Quast, 1937; Phillipson, 1939; Ohga, Ota and Nakazoto, 1965). Phillipson (1939) attributed this association of motility to be artefactual, brought about by the juxtaposition of the two visci. Physical separation of the abomasum and reticulum is a prerequisite to determine if reticulum distension produces a reflex influence on abomasal motility.

Figure 1.

A schematic drawing of the forestomach and abomasum of the sheep as viewed from the right-hand side. The regions are

1. The line of the costrochondral arch.
2. Oesophagus.
3. Rumen (dorsal sac).
4. Rumen (ventral sac).
5. Reticulum.
6. Omasum.
7. Abomasal body.
8. Abomasal antrum.
9. Pylorus.
10. Duodenal bulb.
11. Incisura Angularis.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AIMS OF THE EXPERIMENTS.

Perusal of the literature reveals that much is known about neural mechanisms involved in the motility of the monogastric stomach. Stomach motility may be affected by excitatory or inhibitory reflex mechanisms mediated through the intrinsic and extrinsic nerves. Unitary discharge correlates in afferent and efferent fibres to many of these reflex mechanisms have been described. Little information is available about reflex mechanisms affecting the motility of the adult abomasum.

A vagal non-cholinergic, non-adrenergic inhibitory innervation of the monogastric stomach has been described. No evidence for a vagal inhibitory innervation of the adult abomasum has been presented.

The discharge characteristics of efferents associated with reticulorumen motility have been described. No description of the discharge characteristics of single efferents proved to be supplying either the monogastric stomach or the abomasum has been found.

The aims of these experiments were:-

a). To identify reflexes that might be functional in the control of abomasal motility, and to determine the neural mechanisms whereby these reflexes are mediated.

b). To look for evidence supporting the concept of a vagal inhibitory innervation to the abomasum.

c). To characterise the discharge activity of the efferent nerves supplying the abomasal antrum.

CHAPTER TWO.

MATERIALS AND METHODS.

1. TECHNIQUES AVAILABLE FOR INVESTIGATION OF NEURAL MECHANISMS IN ABOMASAL MOTILITY.

In order to investigate factors affecting abomasal motility adequate means for assessing motility must be available.

Abomasal motility (or 'motor profile') can be considered as consisting of tonic muscular activity and contractile muscular activity. Both frequency and amplitude of the contractile activity should be considered. The abomasal motor profile may be assessed by radiographic techniques, intraluminal pressure recordings, e.m.g. recordings, serosal force transducer and displacement transducer recordings. Each technique has disadvantages. Direct observation is subjective, non-quantitative and leaves no permanent record for retrospective analysis. Records obtained from radiographic techniques are essentially two-dimensional and cannot be adequately quantified. Intraluminal pressure recordings may reflect pressure changes occurring outside the viscus being studied; they show the net sum of, and can thus mask local variations in, viscus pressure. Also an intra-luminal recording device may evoke reflex responses in the viscus being studied. Electromyographic recordings show muscle electrical activity at discrete loci and, unless an array

of electrodes is used, do not necessarily reflect whole viscus activity. Serosal force transducers measure mainly isometric contraction and displacement transducers measure isotonic contraction. Force and displacement transducers also measure activity at discrete loci.

Techniques for investigating neural mechanisms affecting abomasal motility include electrical stimulation of the peripheral ends of transected nerves innervating the abomasum, recording the discharge activity in the efferent nerves innervating the abomasum, neurotomy, and observation of the effects on abomasal motility of application of mechanical (or other) stimuli to any region of the body. Each of these techniques has inherent disadvantages. Any technique involving extensive surgery and/or anaesthesia must interfere to some extent with normal neural function. Electrical stimulation of the peripheral ends of transected abomasal nerves may evoke responses through antidromic stimulation of afferents. Selective electrical stimulation of functionally similar fibre types is not feasible without pharmacological interference, so the net result on abomasal motility is the sum of simultaneous electrical stimulation of different fibre types. Repetitive electrical excitation of a unit does not mimic the natural discharge patterns of that unit; these discharge patterns may be functionally important (Carr, 1975; Edwards, 1984). Recording of efferent nerve activity by nerve dissection techniques does not allow the determination of efferent destination or function, nor, because of the peculiar anatomy of the nerve supply to the ovine abomasum,

determination of the site of efferent origin. Interpretation of the effect of applied mechanical stimuli and evoked reflexes is hindered by the problem of stimulus specificity. Nevertheless, each technique when used appropriately can provide insight into the functional mechanisms of the neural control of abomasal motility.

2. MATERIALS AND METHODS USED IN THESE EXPERIMENTS.

ANIMALS.

A total of 54 mature Scottish Blackface, Greyface or crossbred sheep (25 - 100 kg) was used. Animals were kept in groups of up to 6 sheep in a loose yard on a diet of hay and barley. Individuals were penned separately for a minimum of twenty four hours before experiments and allowed hay and water ad libitum.

ANAESTHESIA.

Anaesthesia was induced with 4% halothane in a half-and-half oxygen and nitrous oxide mixture given by face-mask. Anaesthesia was maintained by 2-3% halothane in oxygen delivered by means of a to-fro system via an endotracheal tube until insertion of a tracheostomy tube and catheterization of left femoral artery and vein. Thereafter anaesthesia was maintained by 1.0 % chloralose given intravenously at approximately 45^{o}C at an initial dose of 60 mg/kg and thereafter at a rate of 20mg/kg at approximately two-hour intervals, or when appropriate. Anaesthesia was maintained at a level such that panniculus and corneal reflexes were just demonstrable.

MAINTENANCE AND MONITORING OF PHYSIOLOGICAL PARAMETERS.

Body temperature was maintained at 39^{o}C by an electric blanket connected via a CFP Homeothermic Blanket Control unit to a deep rectal thermistor.

Tracheal carbon dioxide concentration was monitored by an infra-red gas analyser (P.K. Morgan PLC.) calibrated over the range 0.05 - 10.50 %. If spontaneous breathing was inadequate the animal was artificially ventilated using a positive pressure respiration pump (C.F. Palmer, PLC) to maintain tracheal carbon dioxide at 2.5 - 4.0%. These figures were derived from the range displayed when the spontaneous breathing of the animal was adequate.

Systemic arterial pressure was recorded from a polythene catheter positioned in the femoral artery, by means of a Statham transducer. Blood pressure was displayed on an oscilloscope (Tektronix Dual Beam Storage) and recorded on heat sensitive paper and F.M. magnetic tape (TEAC R-351F Data Recorder).

The electrocardiogram (e.c.g.) was taken from standard limb leads, displayed on the oscilloscope (Tektronix Dual Beam Storage) and stored on heat sensitive paper and F.M. magnetic tape (TEAC R-351F data Recorder).

Fluid replacement therapy was given at a rate based on the assumption that requirements for adult animals up to 60 kg body weight are equal to 40 ml/kg/day (Hall, 1967). Mammalian Ringers (8.0 g NaCl, 0.2g KCl, 4.0 g $CaCl_2$, 0.1 g $MgCl_2$, 0.05 gNa_2PO_4, 1.0 G $NaHCO_3$, 1.0 g Glucose litre^{-1}

aqueous solution) solution was used. Colloidal fluids (Haemaccel, Hoechst) were given if arterial pressure fell below 75 mmHg.

An appropriate level of surgical cleanliness was maintained during all experimental preparation. Tissue trauma was kept to a minimum.

EXPERIMENTAL DESIGN.

Four experimental preparations were used. Preliminary surgical procedures were the same for each preparation.

a). Preliminary Procedures .

The left femoral artery and vein were cannulated in the inguinal region with heparinized saline-filled (100 units/ml.) wide bore polyvinyl catheters fitted with three way taps.

Forestomach regurgitation was prevented by ligating the oesophagus in the mid-cervical region. A tracheostomy tube was inserted and secured in the trachea for administration of anaesthetic gases and for positive pressure ventilation if required.

To prevent complications from ruminal distension due to accumulation of gas from fermentation some three-quarters of the reticulorumen contents were removed via a rumenotomy in the left sub-lumbar fossa. A wide-bore (6mm internal diameter) catheter was placed through the right dorsal rumen wall to allow escape of gas while the animal was in left lateral recumbancy. All incisions were sutured

after removal of reticulorumen contents.

b. Specific Procedures.

Many of the preparations involved surgical transection of the abomasum to form separate body and antral pouches. Therefore motility patterns of the entire and transected abomasum were compared. Data for this comparison were obtained from all four preparations described below. Spontaneous motility patterns of the entire or transected abomasum were recorded for periods of up to two hours before any attempt was made to modulate abomasal motility.

1. Preparation to investigate the effect of electrical stimulation of the peripheral end of the cut cervical vagus on abomasal motility.

A 4 cm length of either the left or right cervical vagus was freed from the connective tissue attachments of the carotid sheath and transected between two ligatures. The peripheral end of the cut nerve was placed in a trough-shaped piece of rubber tubing and smeared with vaseline to prevent dessication and to give electrical insulation from surrounding tissues. The nerve lay in the notch of a pair of curved stimulating electrodes situated in the hollow of the tubing. The position of the nerve on the stimulating electrodes was adjusted occasionally to ensure good electrical contact.

Nerve stimulation was achieved by connecting the stimulating electrodes to an isolated stimulator (Devices

Mk IV), triggered by a gated pulse generator (Devices) at frequencies determined by the settings on a digitimer (Devices). The nerve was stimulated at constant pulse duration (1 ms). Voltage amplitude (read off the oscilloscope) of up to 50 V were used; this was judged to be supramaximal (Iggo, 1954). Frequencies of stimulation of up to 50 Hz were used as this encompassed the range of discharge rates encountered in abomasal efferent nerve recordings (Chapter 7). To prevent excessive bradycardia, periods of electrical stimulation of the vagus were restricted to 10 s.

Electrical stimulation of the cervical vagus rather than the abdominal vagus was chosen because the vagal and splanchnic supplies to the abomasum share the same nerve trunk at the abdominal level (Habel, 1956). Electrical stimulation of the peripheral end of the vagus was chosen in preference to central end stimulation because :-

a). Central end stimulation may produce reflex effects either via contralateral vagal efferents or via sympathetic efferents. Vago-sympathetic interaction in relation to stomach motility has been described in the ferret (Andrews, Grundy and Lawes, 1980).

b). Central end stimulation may mediate excitatory effects by eliciting an increase in the activity of excitatory efferents or a decrease in the activity of inhibitory efferents. Central end stimulation may mediate inhibitory effects by eliciting a decrease in the activity of excitatory efferents or an increase in the activity of

inhibitory efferents.

Although the interpretation of the results of electrical stimulation of the peripheral end of nerves is fraught with difficulties (see Chapter 4) it was considered less fraught than interpretation of the results of central end stimulation.

The contralateral vagus was kept intact to maintain preparation viability.

With the sheep in left lateral recumbancy a right abdominal paracostal incision was made 4 cm caudal to the last rib from the level of the costrochondral junction to 2 cm lateral to the ventral mid-line on the same side. The abomasum was exteriorized as far as possible without damage to the omental attachments. The exteriorized portion was maintained at body temperature by placing it in a perspex bath heated by a water-jacket. The surface temperature of the exteriorized abomasum was monitored by placing a calibrated thermistor, attached to a digital display unit, on the uppermost abomasal serosa, and used as a feedback control on the waterbath temperature.

The abomasum was transected 2-4 cm cranial to the incisura angularis to form separate body and antral portions. Care was taken not to transect major blood vessels supplying either portion. Abomasal contents were removed. Balloon catheters for the purposes of recording pressure were placed in the abomasal body and antrum and the incisions sutured to form separate body and antral pouches. The antral balloon catheter was anchored in place

by a retaining suture. Body and antral pressure were monitored by attaching the catheters to Statham pressure transducers coupled with an eight-channel pen recorder (Lectromed).

Pressure in a viscus may be measured by using open-tipped catheters, air- or liquid-filled balloon-tipped catheters, or flaccid balloon-tipped catheters. Open-tipped catheters were not used in these experiments for a number of reasons: they require constant flushing; the pressure measured at the catheter tip does not reflect that of the whole viscus; their use requires a closed system and no satisfactory method of closing the omaso-abomasal junction was devised. Air- or liquid-filled balloon catheters were not used as they may cause unwanted stimulation of abomasal mechanoreceptors (Harding and Leek, 1972, 1973). Flaccid balloon catheters were found to provide a sensitive means of recording viscus pressure while minimizing mechanoreceptor stimulation. A large balloon (displacement 115 ml) was used in the body and a smaller balloon (displacement 10 ml) used in the antrum.*

Exposed tissues were covered in clingfilm (Safeways Foodstores Ltd.) to prevent fluid loss and cooling by evaporation.

2.Preparation to investigate the influence of reticulum distension on abomasal body tone.

With the sheep in right lateral recumbency the reticulorumen was emptied via a rumenotomy in the left sub-

lumbar fossa. A 4 cm ventral midline incision was made beginning 3 cm caudal to the xiphoid process. To allow reticulum distension a balloon catheter was placed in the reticulum via the rumenotomy. The ventral 3 cm of reticulum were pushed through the ventral midline incision and the catheter exteriorized via a stab incision in the ventral pole of the reticulum. The stab incision was sutured before returning the reticulum to the peritoneal cavity. This procedure minimized tissue trauma. A similar balloon catheter was placed in the abdominal cavity immediately cranial to the reticulum. Distance markings on the tubing of the reticulum and abdominal balloon catheters allowed monitoring of the balloon catheter position. The ruminotomy and ventral midline incisions were repaired.

The animal was then placed in left lateral recumbency and the abomasum exteriorized as described in the preceding section. A 3-4 cm incision at right angles to the long axis of the abomasum made in the abomasal body wall 2-4 cm cranial to the incisura angularis and the abomasal contents evacuated. A balloon catheter was placed in the abomasal body through the abomasal incision and retained with an anchoring suture. The abomasal incision was closed. Abomasal body pressure was monitored by attaching the balloon catheter to a Statham pressure transducer coupled to an eight-channel pen recorder (Lectromed).

Electromyographical activity was recorded from enamel-coated wires (Stabilohm 110, Johnson Matthey Metals, 0.14 mm diameter) inserted into the muscle coat of the abomasal body using a 21-gauge needle as a guide. The end 5 mm of

wire was stripped of enamel and folded to form a barb which served to hold the wire in place in tissue. Wires were coupled to an eight-channel pen recorder (Lectromed) which displayed both direct and integrated e.m.g. traces.

3. Preparation for investigating the effects of abomasal body distension on the motility of the abomasal antrum.

The abomasum of 19 sheep were exteriorized as previously described. The effect on abomasal antrum motility of inflation of a balloon catheter in the abomasal body was investigated in 5 of the 19 sheep with an intact abomasum and 14 of the 19 sheep with the abomasum surgically transected to form separate body and antral pouches as previously described. A balloon catheter was placed in each of the body and antrum of the intact abomasum through a 3-4 cm dorso-ventral incision in the abomasal body 2-4 cm cranial to the incisura angularis. A balloon catheter was placed in each of the body and antral portions of the transected abomasum prior to the portions being sutured to form body and antral pouches. A retaining suture held the antral balloon in place. The body catheter allowed body distension; the antral catheter, coupled to an 8-channel pen recorder via a Statham pressure transducer allowed antral pressure to be monitored. Body and antral pouches were kept physically separate (by means of either a perspex barrier or two rigidly held vertical metal bars) as much as possible without tearing the omentum or compromising blood supply.

Antral electromyographic activity was recorded as previously described for the abomasal body.

Bilateral cervical vagotomy was performed on 4 sheep with the abomasum transected. The nerves were isolated from the carotid sheaths and cut between two ligatures. Acute bilateral splanchotomy (by electro-cautery) proximal to the coeliac ganglion was attempted in 4 sheep. This was a traumatic technique causing severe hypotension which proved fatal in 3 of the 4 attempts. Abomasal denervation was attempted by transection of the abdominal continuations of the dorsal and ventral vagi at the level of the omaso-abomasal junction.

4.Preparation for recording the unitary activity of the abdominal continuations of the dorsal and ventral vagi.

The abdominal continuation of either the dorsal or ventral vagus was identified and a 3-4 cm length dissected from the lesser omentum close to the abomasal antrum. The nerve trunk was cut transversely within 2 cm of the abomasal antrum. The cut central end was placed in a perspex dissection bath and covered with liquid paraffin to prevent dessication. The nerve was stripped of epineurium and perineurium, and nerve fibres separated using a technique similar to that described by Iggo (1955). Efferent activity of single nerve fibres or small groups of fibres was recorded using bipolar silver electrodes. Nerves were placed across one electrode and a piece of similarily-sized non-nervous tissue was placed on the other. Electrical activity recorded from nerves was differentially

amplified (Amplifier type 3160, Digitimer plc.), displayed on an oscilloscope (Tektronix D13 Dual Beam Storage) and stored on F.M. magnetic tape. (T.E.A.C. R351-F data recorder). The impulse activity of a single unit could be selected from a 2-3 unit recording using the window discriminator of a spike processor (Digitimer D.130).

Conduction velocity was measured by the peripheral stimulus technique (Iggo, 1958) using Devices Mark IV isolated stimulators triggered from a gated pulse generator (Type 2521, Devices). Conduction distances were derived by measuring the length of a cotton thread which was placed accurately along the nerve between stimulating and recording electrodes. Stimulating electrodes were sited 4-8 cm proximal to the recording electrodes.

To allow off-line analysis of the unitary discharge for cardiovascular and/or antral motility rhythms, spontaneous discharge activity, e.c.g. activity and antral e.m.g. activity were recorded simultaneously on F.M. magnetic tape (TEAC R-351 Data Recorder) for 100-300 s before modulation of the unitary discharge was attempted.

Modulation of unitary discharge was attempted by application of three stimuli.

1. Stimulation of arterial baroreceptors by an intra-venous injection of 100 ug of adrenaline B.P. in 5 ml of normal saline. Iggo and Vogt (1956) showed in the cat that the inhibitory effect on sympathetic discharge of intra-venous injection of adrenaline is due to its pressor

effect as intravenous adrenaline does not affect sympathetic discharge after baroreceptor denervation.

Units were classified into groups according to their peak response to increase in arterial pressure. The parameters of each induced arterial pressure change were examined to ensure that differences in unitary response were not due to differences in stimuli. For this purpose arterial pressure increase was recorded and percentage arterial pressure increase (the resting systolic pressure being taken as 100%) and rate of arterial pressure increase derived.

2. Stimulation of antral mechanoreceptors by inflation of a balloon in the antral pouch with 5-50 ml of air. Inflation was maintained for at least 30 s. Volumes greater than 50 ml were not used as they were judged to cause too severe a distension of the antral pouch.

3. Stimulation of abomasal body mechanoreceptors by inflation of a body pouch balloon with 10-100 ml of air. Volumes of inflation greater than 100 ml usually resulted in displacement of the nerve from the recording electodes. On one occasion inflation of the body pouch to 250 ml was achieved without disturbing the nerve on the recording electrodes. Inflations were maintained for at least 30 s.

The abomasum was exteriorized and surgically transected to form separate body and antral pouches as described above. Body and antral pressures could be monitored by attaching the balloon catheters to Statham pressure transducers coupled to an eight-channel pen recorder (Lectromed).

DATA COLLECTION.

1.Cardiovascular and abomasal motility data.

Systemic arterial pressure, the electrocardiogram (e.c.g.), reticular pressure, abomasal body and abomasal antrum pressure, and body and antrum e.m.g. (direct and integrated) could be collected on heat-sensitive paper by an 8-channel pen recorder (Lectromed).

The e.c.g., abomasal antrum direct e.m.g., arterial pressure, and abomasal body and antral pressure could be collected on F.M. magnetic tape (T.E.A.C. R351-F data recorder).

2.Efferent Data.

After amplification unitary discharge activity was recorded directly onto F.M. magnetic tape (T.E.A.C. R351-F data recorder). Impulse frequencies were counted by a spike processor (D.130, Digitimer Ltd.) and converted into a standard pulse. An integrated trace of impulse frequency was recorded on heat-sensitive paper by an 8-channel pen recorder (Lectromed). Pulses were suitable for on-line and off-line collection by appropriate computer programmes (with Cromemco System Three computer, disc drive, Lear Seigler and Kaga display units, and Epson FX-100 printer). Two collection programmes were used: one program (RATE3F, compiled by Dr A.D. Short, Faculty of Veterinary Medicine, University of Edinburgh) allowed simultaneous collection and display of impulse frequency and up to three analogue

channels; the second (GSPIKE2, compiled by Mrs G. McConnell, Faculty of Veterinary Medicine, University of Edinburgh) collected interspike and intertrigger intervals on the same time base for off-line post stimulus time histogram analysis.

DATA ANALYSIS.

1. General.

a. Abomasal pressure recordings.

Body and antral pressure were analysed by hand from recordings made on heat sensitive paper. Intraluminal pressure was considered as composed of 'tone' with superimposed 'contractions'. Contraction amplitude was taken as the distance from trough to following peak on the pressure trace. The tone of a viscus was defined as the pressure existing in the viscus in the absence of superimposed contractions. If the viscus was contracting, the mean level of the troughs of the pressure trace was taken as the tone value (fig. 2, 3).

b. Electromyographic recordings.

Raw and integrated e.m.g. recordings were not analysed quantitatively. Changes in e.m.g. activity were often apparent on the trace, and where the direction of change was obvious, were classified as 'activity increase' or 'activity decrease'. The magnitude of e.m.g. change often did not reflect changes in viscus activity that were apparent on the pressure trace and by observing the viscus

(fig. 4). This was ascribed to the fact that e.m.g. electrodes sample muscle electrical activity at very discrete loci and, unlike a balloon, do not necessarily reflect the activity of the viscus as a whole. As the emphasis in these experiments was on changes in whole viscus activity interpretation was based mostly on recorded changes in viscus pressure.

2. Analysis of data derived from the intact and transected abomasum.

The spontaneous frequency of contraction, tone, and amplitude of contraction of the body and antrum of the entire and transected abomasum were compared. Amplitude of contraction of a preparation was taken as the mean of 10 contractions selected by a random numbers method from that preparation. The body and antral frequency of contraction, tone and amplitude of contraction of the entire and transected preparations were compared by a one way analysis of variance and Student's t-test.

3. Analysis of data concerned with the effect of reticular distension on the tone of the abomasal body.

The effects of inflation of intra-reticular and intra-abdominal balloons on abomasal body tone in each preparation were compared using a one way analysis of variance and Student's t-test. To enable group analysis of the experiments, changes induced in body pressure by inflation of either balloon were normalized.

4. Analysis of data concerned with the effect on antral motility of inflation of a balloon in the abomasal body.

The effect of inflation of a body balloon on the motor profile of the abomasal antrum was judged by comparing the amplitude of the 10 antral contractions immediately preceding body inflation with the amplitude of the 10 antral contractions immediately following body inflation. Significance was determined using a one way analysis of variance and Student's t-test.

5. Analysis of data derived from efferent recording experiments.

a. Rate Analysis.

Spike trains were analysed for correlation between changes in discharge rate and the magnitude of applied stimuli. Stimuli were as follows: activation of arterial baroreceptors by increasing blood pressure; stimulation of abomasal body mechanoreceptors by body inflation; stimulation of abomasal antral mechanoreceptors by antral inflation.

Impulse frequency s^{-1} over any given period could be determined using a computer programme (RATSTATF, compiled by Mrs G. McConnell, Faculty of Veterinary Medicine, University of Edinburgh) compatable with the RATE3F collection programme described above. Spontaneous discharge rate was compared with discharge rate over the 10 s period of peak induced arterial pressure rise. The correlation between the percentage change in discharge rate

and percentage changes in arterial pressure and rate of change of arterial pressure was examined.

Inflation of the body pouch resulted in a 'peak and plateau' in the abomasal body trace as the body relaxed to accommodate the increased volume. Using the RATSTATF programme the discharge rate during the plateau phase induced by any volume of inflation of the body pouch was compared with the discharge rate when the body pouch was empty.

b. **Post Stimulus Time Histogram analysis.**

Spike trains were analysed for temporal relationships with the e.c.g and antral e.m.g. by constructing post stimulus time histograms with respect to the R-wave of the e.c.g. and the start of the electrical spiking activity (e.s.a.) of the antral e.m.g. respectively. For this analysis a computer programme was used (GPSTH, compiled by Mrs G. Mc Connell, Faculty of Veterinary medicine, University of Edinburgh). Inter R-wave intervals were measured by using the R-wave to trigger a standard pulse by means of the window discriminator of a Digitimer D.130 Spike processor. Inter-R-wave intervals were divided into bins of 10 ms. The window discriminator could not be used to measure antral e.m.g. periodicity because of the irregular shape and amplitude of the electrical spiking activity of the antral e.m.g. Therefore the antral e.m.g. was displayed on an oscilloscope (Tektronix Dual Beam) and a standard pulse triggered manually at the start of the e.s.a. of each antral e.m.g. cycle. The manual trigger was

also displayed on the oscilloscope. The maximum observed delay between the start of antral e.s.a. and the trigger pulse was 300 ms. As the inter-trigger interval was divided into bins of 100 ms and the shortest interval encountered between trigger pulses was 5000 ms, the maximum error introduced by the manual trigger technique was 6 %. This degree of error was felt to be acceptable for the purposes of this PSTH analysis.

CHAPTER THREE.

MOTILITY PATTERNS IN THE INTACT AND TRANSECTED ABOMASUM.

INTRODUCTION.

Quantitative analyses of the motor profile of the adult ovine abomasum have been limited to consideration of the e.m.g. activity (Ruckebusch, 1970; Ruckebusch and Bueno, 1977). Detailed analysis of adult ovine abomasal pressure fluctuations has not been made. In a study of the abomasal motor profile consideration must be given to both tonic and contractile muscular activity. Also, because of the structural and proposed functional differences between the two regions individual consideration should be given to the abomasal body and abomasal antrum. This chapter considers the motor profile of the body and antrum in acute preparations with the abomasum either intact or transected, and assesses the suitability of such preparations for examination of the neural mechanisms of abomasal motility.

Independent abomasal body and abomasal antrum (henceforth referred to as 'body' and 'antrum') pressure recordings were taken in forty-two sheep, twenty-one of which had the abomasum surgically transected to form separate body and antral pouches. Activity patterns are descibed. The frequency of contraction, amplitude of contraction and baseline pressure ('tone') in intact and transected abomasal preparations were compared.

RESULTS.

The motor profile of the abomasal body

The motor profile of the abomasal body wall was composed of a prevailing tone upon which contractile activity could be superimposed. Three patterns were seen.

1. 'Non-contractile'. The body motor profile consisted of tonic muscular activity only.

2. 'Periodic'. The body motor profile consisted of tonic muscular activity upon which periods of contractile activity were superimposed.

3. 'Continuous'. The body motor profile consisted of tonic muscular activity upon which contractile activity was always superimposed.

Body tone in transected preparations (mean = 1.6 mmHg, standard deviation (s.d.) = 1.5 mmHg) was significantly lower than body tone in intact preparations (mean = 3.2 mmHg, s.d. = 1.8 mmHg).

Contractile activity of the body varied from non-existent through periodic to continuous (fig. 2) in both transected and intact preparations. In the sample of 42 sheep the body was non-contractile on 25 occasions (13 in transected preparations), showed periodic contractile activity on 5 occasions (2 in transected preparations) and continuous contractions on 12 occasions (6 in transected preparations). When contractile activity was periodic, it could occur as isolated contractions or bursts of 1-60 min

duration. Inter-burst intervals were of 1-60 min duration.

Amplitude of body contraction in transected preparations (mean = 0.7 mmHg, s.d. = 0.5 mmHg, n = 80 contractions from 8 sheep) did not differ significantly from the amplitude of body contraction in intact preparations (mean = 0.8 mmHg, s.d. = 0.7 mmHg, n = 90 contractions from 9 sheep). The range of body contraction amplitudes was 0.1-4.8 mmHg.

In preparations where body contraction occurred, frequency of body contraction in transected preparations (mean +/- s.d. = 5.3 +/- 0.7 min^{-1}, n = 8) did not differ significantly from the frequency of body contraction in intact preparations (mean +/- s.d. = 4.9 +/- 1.1 min^{-1}, n =9). The range of frequency of body contraction was 4-7 min^{-1}.

Body e.m.g electrical spiking activity occurred in phase with abomasal body contractile activity.

Motor profile of the abomasal antrum.

There was no significant difference between the tone of the antrum in transected and intact preparations (mean +/- s.d. = 2.4 +/- 2.1 mmHg and 3.7 +/- 2.5 mmHg respectively n = 2I in each case).

The patterns of antral contractile activity were similar in the transected and intact abomasum. With the exception of two experiments (one transected, one intact) where the antrum was quiescent, contractile activity in the antrum was continuous. Observation of the contractions suggested that they were peristaltic in nature, although in two

transected preparations anti-peristaltic waves were observed. Antral contraction amplitude could be more-or-less constant for the duration of recording or could be irregular. In one transected preparation antrum contraction amplitude waxed and waned **regularly** over a cycle of 5-10 contractions. In others a pattern of regular contraction amplitude broken by bursts of higher amplitude contractile activity at irregular intervals was seen. These bursts of high amplitude activity lasted for 1-10 min. (fig. 3). Antral contraction amplitude in transected preparations (mean +/- s.d. = 2.8 +/- 2.4 mmHg, n = 100 from 10 sheep) was significantly greater than that of intact preparations (mean +/- s.d. = 1.8 +/- 1.1 mmHg, n = 100 from 10 sheep).

Frequency of antrum contraction in transected preparations (mean +/- s.d. = 5.1 +/- 0.2 min^{-1}) did not differ significantly from the frequency of antrum contraction in intact preparations (mean +/- s.d. = 5.4 +/- 0.9 min^{-1}).

The records of antrum e.m.g. activity showed electrical spiking activity (e.s.a.) coincident with antral contractile activity (fig. 3, 4). Changes in amplitude of antral contraction were not always reflected by similar changes in e.m.g. activity (fig. 4). Patterns of e.m.g. activity in transected preparations did not differ from that of intact preparations.

Comparison of abomasal body and abomasal antrum parameters.

Of the intact preparations, body tone was less than antral tone in 65% of preparations (mean difference +/-

s.d. = 1.4 +/- 0.7 mmHg), equal to antral tone in 10% of preparations and greater than antral tone in 25% of preparations (n = 20). In the transected preparation the corresponding figures were 65% (mean difference +/- s.d. = 1.3 +/- 1.0 mmHg), 25% and 10% (n = 20).

In preparations, transected or intact, in which both body and antrum were contracting, the mean amplitude of body contractions was always less than the mean amplitude of antrum contractions.

DISCUSSION.

Body motility.

The patterns of body motility described in acute and chronic preparations (Czepa and Stigler, 1929; Kryzwanek and Quast, 1937; Phillipson, 1939; Stevens, Sellers and Spurrell, 1960; Ohga, Ota and Nakazoto, 1965; Ruckebusch, 1970; Ruckebusch and Bueno, 1977; Bell, 1978; Reid, Schulkes and Titchen, 1988a, 1988b; Reid and Titchen, 1988), have also been seen in these experiments. The range of frequency of body contraction in these experiments (4-7 min^{-1}) is less than that described by Reid, Shulkes and Titchen (1988a) for the body of the anaesthetized lamb (4-17 min^{-1}), or the conscious preruminant calf (Bell and Grivel, 1975). This may be due to differences in the neural control mechanisms of the abomasum of adult and immature ruminants. Anaesthesia may affect the range of contraction frequencies exhibited by the body. Reid, Shulkes and Titchen (1988b) found the range of frequency of contraction

of conscious lambs to be 6-12 min^{-1} and 4-17 min^{-1} in pentobarbitone - anaesthetized lambs (Reid, Schulkes and Titchen 1988a). Classically, the frequency of contraction of the abomasum is considered to be independent of extrinsic neural control. Thus it is possible that the effect of anaesthesia on the frequency of body contraction is at the muscle rather than the neural level.

No reference to quantification of the amplitude of contraction or the tone of the body or the antrum of the chloralose-anaesthetized adult sheep has been found. In this acute preparation both body tone and amplitude of body contraction were usually lower than the equivalent value in the antrum. In acute invasive preparations such as this, where the animal is anaesthetized, where exact information on the feeding behaviour immediately preceding the experiment is not available, where the functional pattern of forestomach motility has been disrupted by rumenotomy and where the functional pattern of abomasal motility has been interrupted by emptying the abomasum it is unwise to place too much functional significance on observations of this nature. If in the conscious animal the same differences exist in amplitude of contraction and tone between the body and antrum as were found in this preparation the functional implication is that movement of digesta from the abomasal body to the abomasal antrum cannot rely on either the prevailing pressure gradient or the force of abomasal body contractions. Thus a supplementary mechanism may exist for the transfer of digesta from the abomasal body to the abomasal antrum.

Gravitational flow augmented by the movements of adjacent visci were suggested by Phillipson (1939) and this is one possibility. Gravitational forces working in the standing animal were not working in the acute preparation as, with the animal in left lateral recumbancy , body and antrum were at the same vertical elevation. The contractions of the antrum acting as a pump on digesta pushed toward the antrum by incoming digesta from the forestomach , so moving digesta from the abomasal body toward the pylorus is another possible mechanism. Developing this latter suggestion, it is reasonable to propose that an increase in abomasal body distension elicits an increase in activity of this antral 'pump'. The possibility of such a mechanism has been investigated (Chapter 6). Incoming digesta from the forestomach may also act as a stimulus to abomasal motility.

In 90% of untransected preparations abomasal body tone differed from abomasal antral tone. It is interesting to postulate on how a tone difference is maintained between two parts of what is essentially a single hollow viscus. The mucosal leaves of the distal abomasal body and the incisura angularis may combine to form some sort of functional barrier between the abomasal body and the abomasal antrum. The apparent difference in body and antral tone may have been due to the difference in the flaccidity of the body and antral balloons.

The pattern of continuous contraction observed in the abomasal antrum is similar to that described in the pentobarbitone-anaesthetized lamb (Reid, Shulkes and Titchen, 1988a). This pattern of continuous antral activity differs from that described in conscious animals where, at intervals of 60-90 min, 10 min periods of antral quiescence occur coincident with bursts of regular spiking

activity of the duodenal e.m.g. (Ruckebusch and Bueno, 1977). The inference is that anaesthesia at least partly disassociates the co-ordination between the abomasal antrum and the duodenum. Thus the value of anaesthetized animals as a model for studying mechanisms of abomaso-duodenal interaction is questionable.

Comparison of the intact and transected preparations

Patterns of activity, tone, and frequency and amplitude of contraction in the body and antrum were compared in transected and intact preparations. The preparations differed on two parameters. Amplitude of antral contraction was greater, and the body tone was lower in transected preparations. The apparent increase in antral contraction amplitude may have been due to the change in recording conditions brought about by transecting the abomasum; the recording balloon was enclosed in a smaller space and pressure fluctuations brought about by abomasal antral contraction were not damped in the 'sink' of the adjacent abomasal body. If this were the case it is reasonable to suppose that an increase in amplitude of contraction would be seen in both body and antrum. In fact transection brings about a non-significant reduction in the amplitude of body contractions. Thus it is more likely that abomasal transection increases the amplitude of antral contraction because in the intact viscus the body exerts an inhibitory influence on the amplitude of antral contractions via the intramural plexi. The reduction in body tone brought about by abomasal transection cannot be due only to an inhibitory

input elicited by the surgical procedures involved as similar effects were not induced in antral tone or in the amplitude of contraction of either body or antrum. The reduction in body tone brought about by transection of the abomasum may be due to the resulting increase in amplitude of contraction of the antrum inducing a fall in body tone via an extrinsic reflex. Alternatively the reduction in body tone brought about by transection of the abomasum may be due to interruption of an excitatory drive to body tone from the antrum mediated through the intramural plexi.

These phenomena, which suggest an excitatory drive to body tone from the antrum and an inhibitory drive to the amplitude of antrum contraction from the body, have been observed when both the body and antrum are empty. As the body fills it is likely that the inhibitory drive to antral contractions is removed: as the amplitude of antral contractions increases it is likely that the excitatory drive from the abomasal antrum to abomasal tone is removed. These mechanisms would help to explain the ability of the abomasum to co-ordinate its motility subsequent to external denervation (Duncan, 1953; Gregory, 1982, 1984).

The patterns of motility in transected and untransected preparations did not differ, and with the exceptions of amplitude of abomasal antrum contraction and abomasal body tone, the parameters displayed by the two preparations were similar. It is suggested therefore that the transected abomasal preparation provides a suitable model for investigation of mechanisms of control of abomasal motility.

Figure 2.

The different patterns of body pouch motility, recorded by flaccid balloon-tipped catheters, seen in the acutely prepared transected abomasum.

A:- the pressure trace of a non-contractile abomasal body. The pressure fluctuations ($17\ min^{-1}$) are due to respiratory movement.

B:- the pressure trace of an abomasal body that contracted periodically. The rapid pressure fluctuations ($17\text{-}18\ min^{-1}$) are due to respiratory movement.

C:-the pressure trace of an abomasal body that contracted continuously at a rate of $5\ min^{-1}$.

Similar patterns of activity were seen in the body of the acutely prepared intact abomasum.

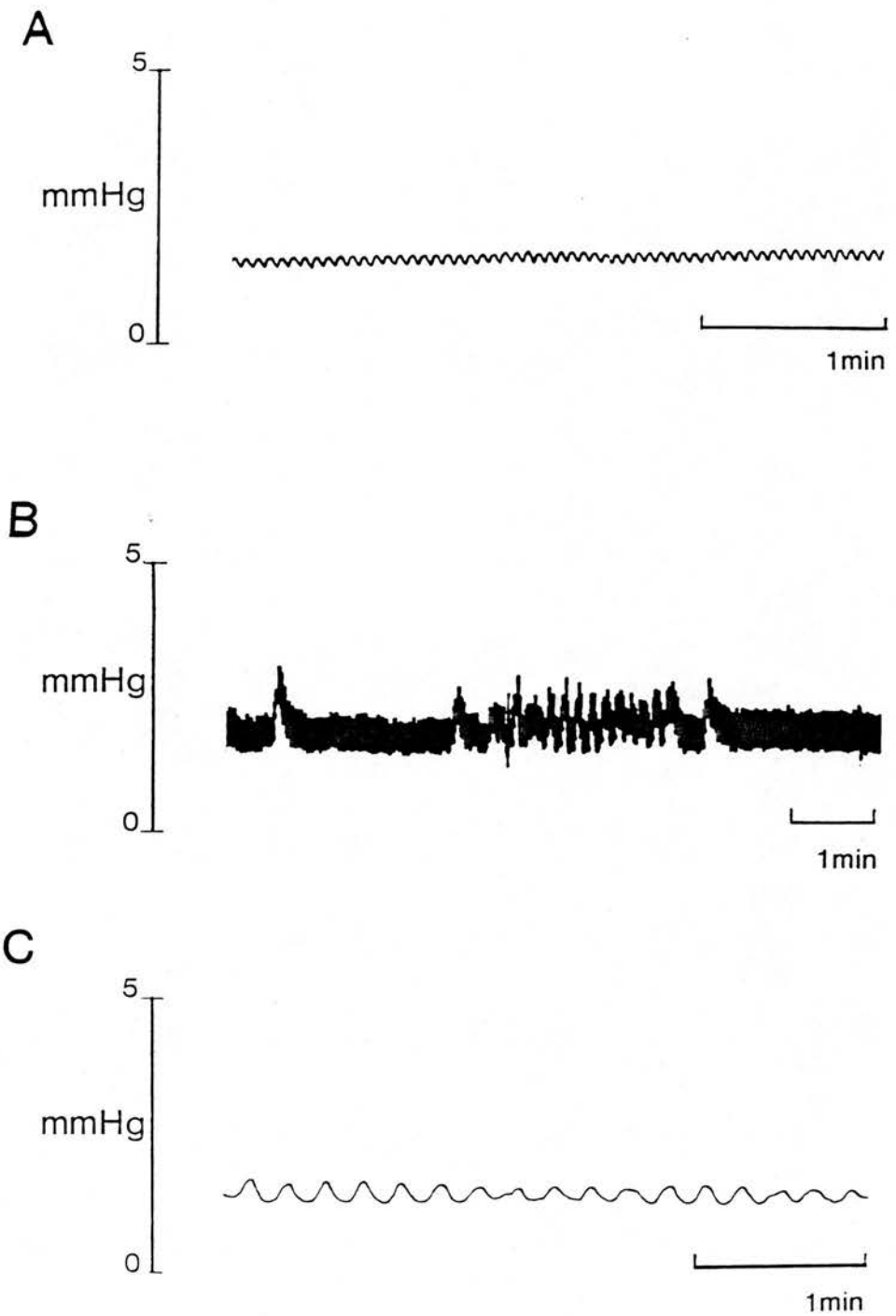

A
5
mmHg
0
1min
B
5
mmHg
0
1min
C
5
mmHg
0
1min

Figure 3.

The patterns of abomasal antral pouch motility observed in the acutely prepared transected abomasum. In each of A, B and D the upper trace is the direct antral e.m.g. and the lower trace is antral pressure, recorded by a flaccid balloon-tipped catheter. In C only the pressure trace is shown.

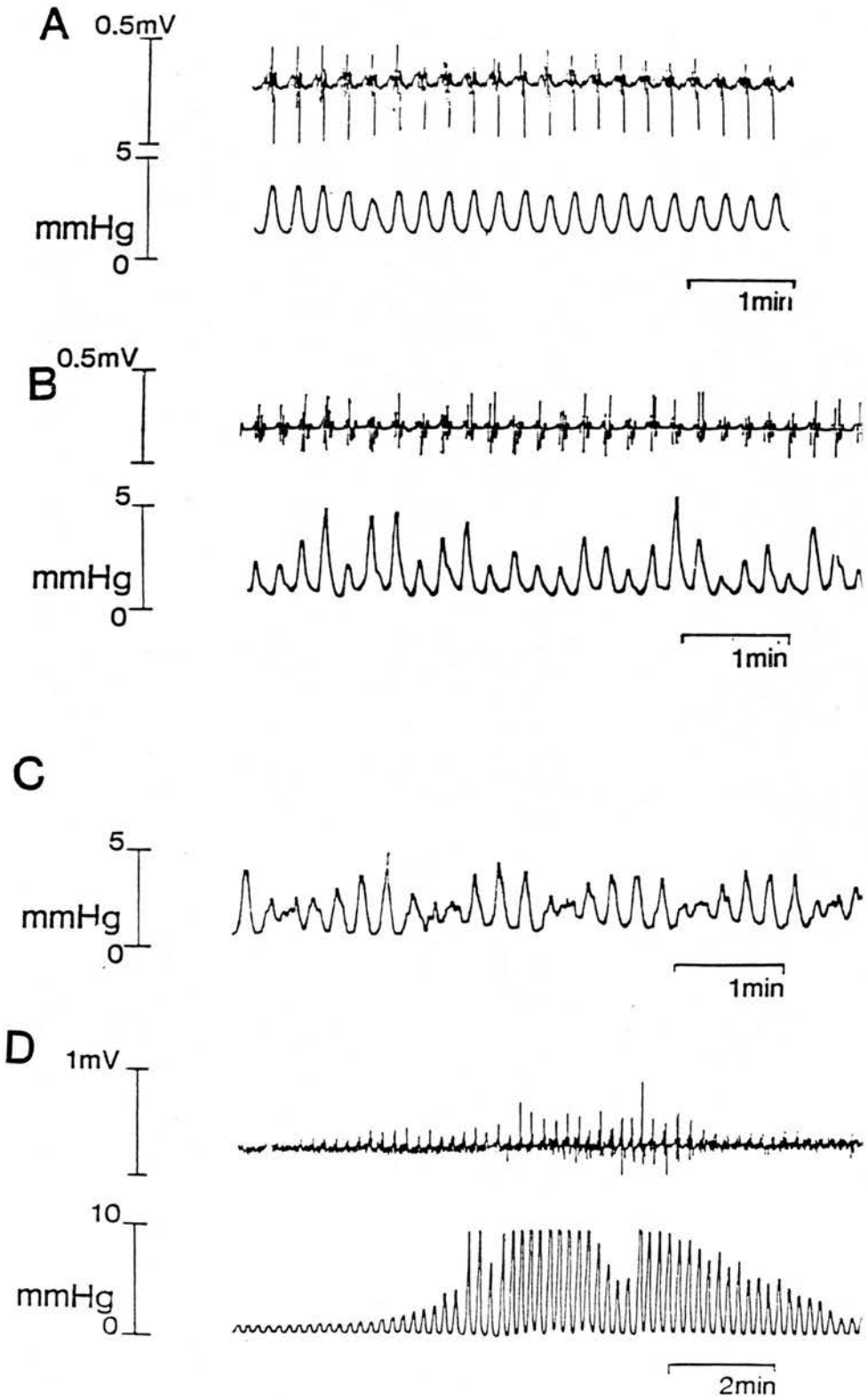
A:- A preparation with a regular amplitude of abomasal antral contraction at a rate of 4.5 min^{-1}.

B:- A preparation with an irregular amplitude of abomasal antral contraction at a rate of 5.0 min^{-1}.

C:- A preparation where the amplitude of abomasal antral contraction waxed and waned at a rate of 5 min^{-1}.

D:- A preparation where the regular amplitude of contraction was interrupted by bursts of higher amplitude contractions both at a rate of 5 min^{-1}.

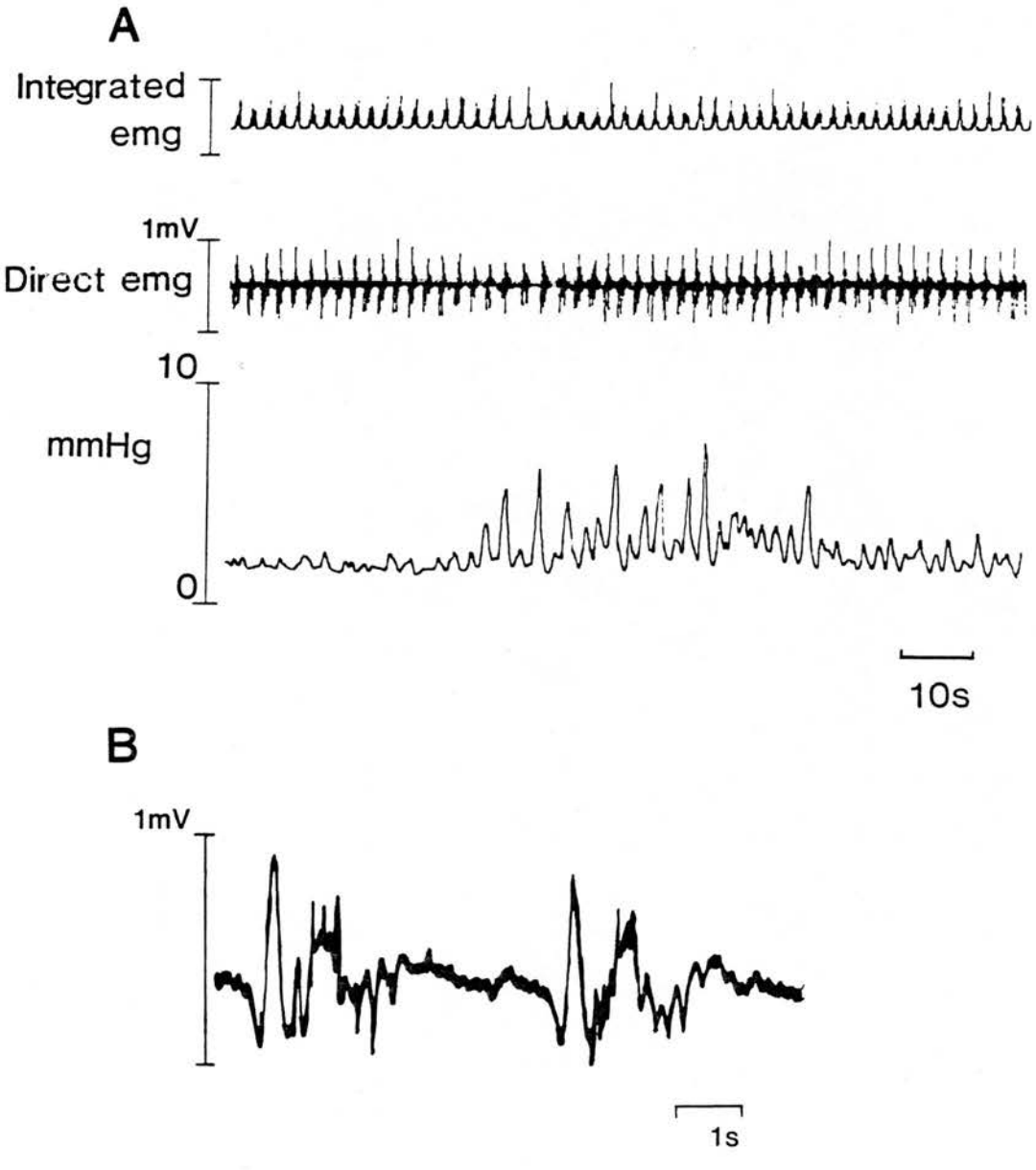
Similar patterns of antral motility were seen in the acutely prepared intact abomasum.


A
0.5mV
5
mmHg
0
1min
B
0.5mV
5
mmHg
0
1min
C
5
mmHg
0
1min
D
1mV
10
mmHg
0
2min

gure 4.

A. Changes in the amplitude of antral contraction flected by the pressure recording (lower trace) were not ways mirrored in the direct (middle trace) or integrated pper trace) e.m.g. recordings. This may be because the m.g. measures the electrical activity of muscle at a ngle discrete locus, while the pressure trace reflects e motor activity of the whole viscus.

B. An expanded portion of direct antral e.m.g. recorded th extra-cellular electrodes shows it to consist of riods of electrical spiking activity separated by periods electrical quiescence.


A
Integrated
emg
1mV
Direct emg
10
mmHg
0
10s
B
1mV
1s

CHAPTER FOUR.

THE EFFECT ON THE ABOMASAL MOTOR PROFILE OF ELECTRICAL STIMULATION OF THE PERIPHERAL END OF THE CUT CERVICAL VAGUS.

INTRODUCTION.

Electrical stimulation of the peripheral end of the cut cervical vagus has identified excitatory and inhibitory components to the vagal innervation of the stomach of monogastric animals (Martinson and Muren, 1963; Paton and Vane, 1963; Bulbring and Gershon, 1967; Burns and Reinke, 1971; Andrews and Scratcherd, 1980; Andrews and Lawes, 1985a, 1985b; Ohta, Nakazato and Ohga, 1985). There has been no comparable study on the effects of electrical stimulation of the peripheral end of the cut cervical vagus on the motor profile of the adult ruminant abomasum. In view of the different mechanisms of digestion in adult ruminant and monogastric animals there is scope for such a study. In particular there is no description of a vagal inhibitory innervation to the abomasum of the adult ruminant, although in atropinized, milk-fed lambs Reid, Schulkes and Titchen (1988a) found that electrical stimulation of the peripheral end of the cut cervical vagus may inhibit the e.m.g. activity of both body and antrum.

In view of the different effects of electrical stimulation of the peripheral end of the cut cervical vagus on the body and antrum of the ferret stomach (Andrews and Scratcherd, 1980) it was felt that separate consideration

of the effects of such stimulation on the abomasal body and antrum motility was appropriate. Most previous accounts of such work in the monogastric animal have considered the stomach as a single functional compartment.

The results of a quantitative study of the effect of electrical stimulation of the peripheral end of the cut cervical vagus on the motor profile of the abomasal body and antrum are described and discussed here. To facilitate differentiation of body and antral responses the abomasum was surgically transected and sutured to form two separated pouches.

PROTOCOL.

The effects on the motor profile of the abomasal body and the abomasal antrum of repetitive electrical stimulation of the peripheral end of either the right (n=5) or left (n=2) cut cervical vagus (henceforth referred to as 'stimulation') in seven sheep were examined over voltage and frequency ranges of 1-50 V and 1-50 Hz respectively. Stimulus pulse width was of 1 ms duration. Pulses were applied for a 10 s period. Interstimulus intervals were variable, but of at least 100 s duration. Stimulus parameters within the stated ranges were altered in random order.

RESULTS.

Frequency- and voltage-response curves were constructed where appropriate for the changes in the motor profile of the body (fig. 5) and antrum (fig. 6) for each preparation.

Body excitatory, body relaxatory and antral excitatory responses were elicited by stimulation of either left or right vagus. Antral inhibitory responses were only seen on stimulation of the right vagus.

Changes in the motor profile of the abomasal body in response to electrical stimulation of the peripheral end of the cut cervical vagus.

Lower frequencies (1-10 Hz) of stimulation of the left or right vagus, providing the stimulus was of sufficiently high voltage, produced one of the following effects: relaxation of the body concurrent with stimulation; no reaction of the body during stimulation but a contraction ('rebound contraction') of the body immediately stimulation stopped; relaxation of the body concurrent with stimulation followed by a rebound contraction of the body immediately stimulation stopped (fig. 7). The period between the start of stimulation of the vagus and relaxation of the body ('relaxation latency') was between 1 and 2 s in all preparations. (Mean +/- s.d. = 1.6 +/- 0.2 s). Relaxation did not persist beyond the period of stimulation. Once the threshold voltage was reached, further increases in voltage did not necessarily increase the degree of body relaxation. In two cases (fig. 5) stimulation of the vagus at low frequencies produced body relaxation at low voltages and body contraction at higher voltages. The maximum frequency tested which produced body relaxation was 5 Hz in two animals and 10 Hz in three animals. In the two animals in which relaxation of the body was not seen, rebound contractions occasionally occurred

after low frequency stimulation. The magnitude of relaxation was small in all preparations (0.1-2.0 mmHg).

Stimulation of either right or left vagus at higher frequencies (10-50 Hz) caused body contraction, providing the stimulation was of a sufficiently high voltage (fig. 8). The magnitude of body contraction increased up to a plateau with increase in applied voltage (fig. 5). Maximum contraction amplitude was achieved at a stimulation frequency of 30 Hz in each preparation. At this frequency a stimulation voltage of 20-30 V often produced a larger body contraction than a stimulation voltage of 50 V. The interval ('contraction latency') between the start of stimulation and the start of body contraction in any preparation was longer than the relaxation latency. The range of contraction latencies for the preparations was 1-5 s (mean +/- s.d. = 2.8 +/- 1.0 s).

A small fall in body pressure preceded the majority of the body contractions induced by higher frequency stimulation of the vagus, but only in preparations where body relaxation could be induced by low frequency stimulation of the vagus (fig. 8). The latencies of these 'pre-contraction relaxations' (1-2 s) were similar to the latencies of the relaxatory responses of the body seen with low frequency stimulation of the vagus.

Body contraction induced by electrical stimulation of the peripheral end of the cut cervical vagus ended in one of four ways (fig. 8):-

1. Pressure returned directly to a steady level.

2. Pressure fell below prestimulus levels and recovered to a steady level over 15-60 s. Contractions were often superimposed on the post-contraction relaxation.

3. Pressure fell to a steady level in two phases; a fast phase followed by a slow phase of 15-30 s.

4. Post-contraction pressure oscillated for 15-60 s before stabilizing.

Post-contraction relaxation of the body was seen only in three preparations (sheep number 2, 3 and 7). Other post-contraction phenomena were also apparent in these sheep. Which post-contraction event was likely to occur could not be predicted on the basis of the applied stimulus parameters. The duration of the post-contraction relaxation was consistent at 20 s in sheep number 2, but varied between 15 and 30 s in sheep number 3, and between 30 and 60 s in sheep number 7. Neither the duration nor the amplitude (0.1 to 1.0 mmHg) of the post-contraction relaxation bore any relationship to the frequency or voltage of stimulation.

Changes in the motor profile of the abomasal antrum in response to electrical stimulation of the peripheral end of the cut cervical vagus.

The preparations could be divided into two groups on the basis of their response to suprathreshold electrical stimulation of the peripheral end of the cut cervical vagus:-

1. Antral contractions were reduced in amplitude or inhibited completely during stimulation (n = 3, fig. 9).

2. Antral contraction amplitude was increased by stimulation (n = 4, fig. 10).

The reduction of contraction seen in the first group occurred at frequencies of 5-45 Hz and voltages of 1-50 V. Except for the minimum frequency and voltage thresholds no relationship was apparent between the stimulus parameters and the degree of inhibition (which was often complete) of antral contraction. This may have been due in part to the irregularity of spontaneous antral contractions in these preparations. Frequencies below 5 Hz did not affect the antral motor profile of this group. Post-stimulus contractions were often of larger amplitude than pre-stimulus contractions (fig. 9). The increase in post-stimulus contraction amplitude could persist for up to 12 contractions after the end of stimulation.

The frequency- and voltage- response response curves of the second antral group are shown in fig. 6. Electrical stimulation of the peripheral end of the cut cervical vagus at 5-50 Hz and 5-50 V produced an antral contraction in this group. The amplitude of the induced antral contraction increased with increase in applied voltage up to a plateau level (fig. 6) in the same manner as the abomasal body. The largest increase in antral contraction amplitude occurred at frequencies of 30-35 Hz in all preparations (fig. 6). The latency of contraction of the induced antral

contractions (range = 6 s, mean +/- s.d. = 2.7 +/- 0.7 s) was greater within a preparation than the latency of contraction of the similarily-induced body contractions.

The characteristic 'pre-contraction relaxation', as described for the abomasal body, was not observed in the antrum. Post-contraction events were similar to those seen in the body: the pressure returned immediately to steady levels; the pressure fell beneath, before returning to a steady level over a period of 30-60 s; the pressure oscillated for 30-60 s before stabilizing (fig. 10). Post-contraction relaxation of the antrum occurred more than once in only one preparation (sheep number 7): other post-contraction events also occurred in this preparation. Amplitude (0.2 to 1.0 mmHg) and duration (15-70 s) of the post-contraction relaxations in this preparation had no apparent relationship with frequency or voltage of stimulation. Which post-contraction event occurred in the antrum could not be predicted on the basis of applied stimulus parameters.

DISCUSSION.

Interpretation of the effects of electrical stimulation of the peripheral end of mixed nerves on the motor profile of a viscus that has inherent spontaneous motility is difficult for a number of reasons. For example, the spontaneous motility of the viscus may obscure the precise effect of the stimulus; there is simultaneous orthodromic activation of motor fibres which may have diverse and opposing functions; differences in conduction velocity of

activated fibres will result in non-simultaneous arrival of impulses at the target organ, especially, as in these preparations, if the site of nerve stimulation is remote from the target organ; unless previous surgical ablation of afferents has occurred the effects attributed to evoked orthodromic impulses in efferent fibres cannot be distinguished from any effects of evoked antidromic impulses in afferent fibres. If the proportion of afferent and efferent fibres in the sheep vagus is similar to that of the rabbit (Evans and Murray, 1954) and cat (Agostini, Chinnock, De Burgh Daly and Murray, 1957) where 90% of vagal fibres are afferent, this latter criticism may be especially pertinent. Use of pharmacological stimulating and blocking drugs may help in distinguishing the effects of orthodromic efferent and antidromic afferent activation, and in revealing the action of functionally distinct fibre groups. Pharmacological agents were not used in these experiments because of the practical difficulties in maintaining preparation viability. Bearing these criticisms in mind, several conclusions can still be drawn from these experiments. However, comparison of these results with the reported results of electrical stimulation of the peripheral end of the cut vagus on the motor profile of the stomach of monogastric animals is made difficult because few people have made separate consideration of the body and antrum.

Electrical stimulation of the peripheral end of either the right or left cut cervical vagus caused excitation or

inhibition of the muscles of the wall of the abomasal body during the period of stimulation. The inhibitory effect was dominant at lower strengths of stimulation, suggesting that if the relaxation was due to activation of inhibitory efferents they are of lower electrical threshold than the fibres evoking body contraction. This is in contrast to the findings of Martinson (1965a, 1965b) and Jansson and Martinson (1965) who found that the inhibitory fibres required higher threshold electrical stimulation than the excitatory fibres in the vagal supply to the cat stomach, and Andrews and Lawes (1985a) who found no difference in the electrical threshold between the inhibitory and excitatory fibres in the vagal supply to the stomach of the ferret. These may be species differences, partly depending on the ratio of myelinated to unmyelinated fibres in the cervical vagus. The 'pre-contraction relaxation' observed at higher frequency/voltage combinations of electrical stimulation: the pre-contraction relaxation' was of similar latency to, and was seen only in preparations that showed, body relaxation evoked by low frequency/voltage combinations of electrical stimulation. No reference to this 'pre-contraction relaxation' has been found in the literature although it is visible in the published figures of Burns and Reinke (1971) and Ohta, Nakazato and Ohga (1985) who observed the effects of electrical stimulation of the peripheral end of the cut vagus in the rabbit and guinea pig respectively. The relaxatory effect was visible with higher frequency/voltage combinations of electrical stimulation because of the difference in latency of onset

of the relaxatory and excitatory effects. This latency difference may be due to a combination of factors: a difference in conduction velocity of the fibres eliciting relaxation and those eliciting contraction (tying in with the apparent lower threshold of electrical excitation of the fibres eliciting relaxation); a difference in the rate of synaptic or neuromuscular junction events; a difference in the response time of the contractile units to the inhibitory and excitatory neurotransmitters.

A consistent finding in the reports of short-duration electrical stimulation of the cut end of the peripheral vagus eliciting relaxation of the stomach of monogastric animals is a rebound contraction of the stomach on cessation of the stimulus (Bulbring and Gershon, 1967; Burns and Reinke, 1971; Andrews and Grundy, 1981). Although the mechanism whereby rebound contractions is brought about is disputed, and is considered to be non-physiological (Andrews and Grundy, 1981), they are indicative of short-term electrical excitation of vagal non-cholinergic, non-adrenergic inhibitory efferents. Rebound contractions were seen in the sheep following low frequency/voltage electrical stimulation of the peripheral cut end of the cervical vagus in all seven preparations, although in two preparations relaxation of the body was not seen. The absence of overt relaxation in these preparations may have been due to lack of sensitivity of the recording technique, or because the effects of the activated excitatory and inhibitory fibres are equal and opposite at the low frequency/voltage combinations used.

Increase in stimulus voltage at a frequency that elicited only body relaxation did not increase the amplitude of relaxation. This could be due to close grouping of the voltage thresholds of the inhibitory fibres, or equal recruitment of excitatory and inhibitory fibres by increasing voltage.

The body contractions elicited by electrical stimulation of the cut peripheral end of the cervical vagus were similar to stomach contractions elicited by peripheral cut vagus electrical stimulation in monogastric animals. They are presumably the result of activation of cholinergic pre-ganglionic fibres. The fairly abrupt response plateau reached with increasing voltage suggests that the thresholds of vagal excitatory fibres to the abomasum are close-grouped. The fact that a 50 V stimulus often produced a smaller body contraction than a 20-30 V stimulus at the same frequency is unlikely to be due to the presence of a high threshold group of inhibitory fibres as 30 V stimulation is likely to be supramaximal for the fibres in the cervical vagus of the sheep (Iggo, 1954).

Many people have reported that a stomach contraction elicited by electrical stimulation of the cut peripheral end of the vagus is followed by a slow-decaying post-contraction relaxation, and that, in atropinized preparations, stomach relaxations elicited by electrical stimulation of the cut peripheral end of the vagus are similarily slow-decaying (although the stimulation-induced relaxation and post-stimulus relaxation may be separated by

a rebound contraction) (Paton and Vane, 1963; Martinson, 1964, 1965a; Jansson and Martinson,1965; Campbell, 1966; Bulbring and Gershon, 1967; Beani, Bianchi and Crema 1971; Andrews and Scratcherd, 1980; Andrews and Lawes, 1985a, 1985b; Ohta, Nakazato and Ohga, 1985). This post-stimulus relaxation has been ascribed to the action of an inhibitory neurotransmitter of long half-life. However this phenomenon is not universal: Burns and Reinke (1971) found that post-stimulus relaxation was a post-contraction but not a post-relaxation phenomenon in rabbits, and the published figures of Andrews and Scratcherd (1980, fig. 2) show that post-stimulus relaxation was not an entirely consistent feature in the ferret. No evidence was found in the sheep preparations to suggest that the post-contraction relaxation occasionally observed in the abomasal body was due to the action of an inhibitory neurotransmitter. In the three preparations in which post-contraction relaxation did occur, it did not occur consistently, and showed no relation to frequency or voltage of stimulation. It is unlikely that post-contraction body relaxation is brought about by the same mechanism as the body relaxation elicited during stimulation as the latter never persisted beyond the period of stimulation. Reflex arcs within or between organs allow them to adapt quickly and precisely to changing conditions: the concept of the action of a neurotransmitter persisting for up to five minutes after the last impulse in the parent nerve seems contrary to the purpose of nervous control. Long lasting relaxation is more likely to be due to activity in 'reverberation circuits'.

The antral response to vagal stimulation was independent of, and quite different to the body response. No antral relaxation occurred at low frequency/voltage combinations of stimulation; in those preparations where cut peripheral vagus stimulation evoked antral contractions no pre-contraction relaxation was seen; antral contraction latency differed from body contraction latency. The form of evoked antral contraction was similar to that of the body and as such is likely to be due to activation of vagal cholinergic preganglionic fibres. Maximum contraction amplitude in both the body and the antrum was eliciting at stimulus frequencies of around 30 Hz: this compares with maximum stomach contraction elicited by cut peripheral vagal stimulation at frequencies of 5-10 Hz in ferrets (Andrews and Scratcherd, 1980) and cats (Martinson, 1964), 20 Hz in guinea pigs (Paton and Vane, 1963; Beani, Bianchi and Crema , 1971) and 16-32 Hz in rabbits (Burns and Reinke, 1971). Post-contraction antral relaxation was not a feature of the evoked antral contactions. That vagal electrical stimulation can evoke inhibition of antral contraction amplitude was demonstrated in three preparations. Similar inhibition of antral contractions with little change in antral tone elicited by electrical stimulation of the peripheral end of the cut cervical vagus has been reported in the rat (Aihara, Nakomura, Sato and Simpson, 1978) and atropinized ferret (Andrews and Scratcherd, 1980), although Jansson and Martinson (1965) could find no evidence for a vagal inhibitory supply to the antrum of the cat. In common with the proposed vagal inhibition of the abomasal body, the inhibitory effect of vagal stimulation on the antrum

was apparent only during the period of electrical stimulation. There was no evidence in the sheep for the release by electrical stimulation of the cut peripheral end of the vagus of an inhibitory neurotransmitter with persistent action.

These experiments provide no indication of the nature of any neurotransmitters involved in the proposed vagal inhibitory innervation of the abomasum. It is interesting to postulate as to whether the body and antrum inhibitory neurotransmitters are the same. Unless the body and antral vagal inhibition is mediated by different neurotransmitters the different actions of the vagal inhibitory innervation on the body and antrum must be inherent properties of the contractile units of the two regions. Such questions cannot be answered until techniques in neurotransmitter functional identification improve.

There is no obvious reason why similar electrical stimulation of the peripheral end of the cut cervical vagus should increase antral contraction amplitude in one group of sheep and inhibit antral contraction in another, ostensibly identical group of sheep. One possibility is that the impulses elicited in the pre-ganglionic fibres by electrical stimulation of the vagus undergo integration at the intramural level. Examples of post-synaptic integration of electrically-excited vagal impulses to the stomach are the post-activation potentiation described by Blair, Harper, Kidd and Scratcherd (1959) in the cat and the refractory period of the ferret stomach described by

Andrews and Grundy (1980). Antral inhibitory responses in the sheep were seen only in preparations in which the right vagus was stimulated; excitatory responses of the antrum were seen with both right and left vagal stimulation. It is likely that this was due to the small numbers of animals used in the trial. It is possible that variation in the proportion of inhibitory and excitatory fibres between the right and left vagus in different sheep may account for the difference in the response of the two groups. This is unlikely as such a variation would probably lead to a gradation of response between animals rather than two oppositely polarized groups.

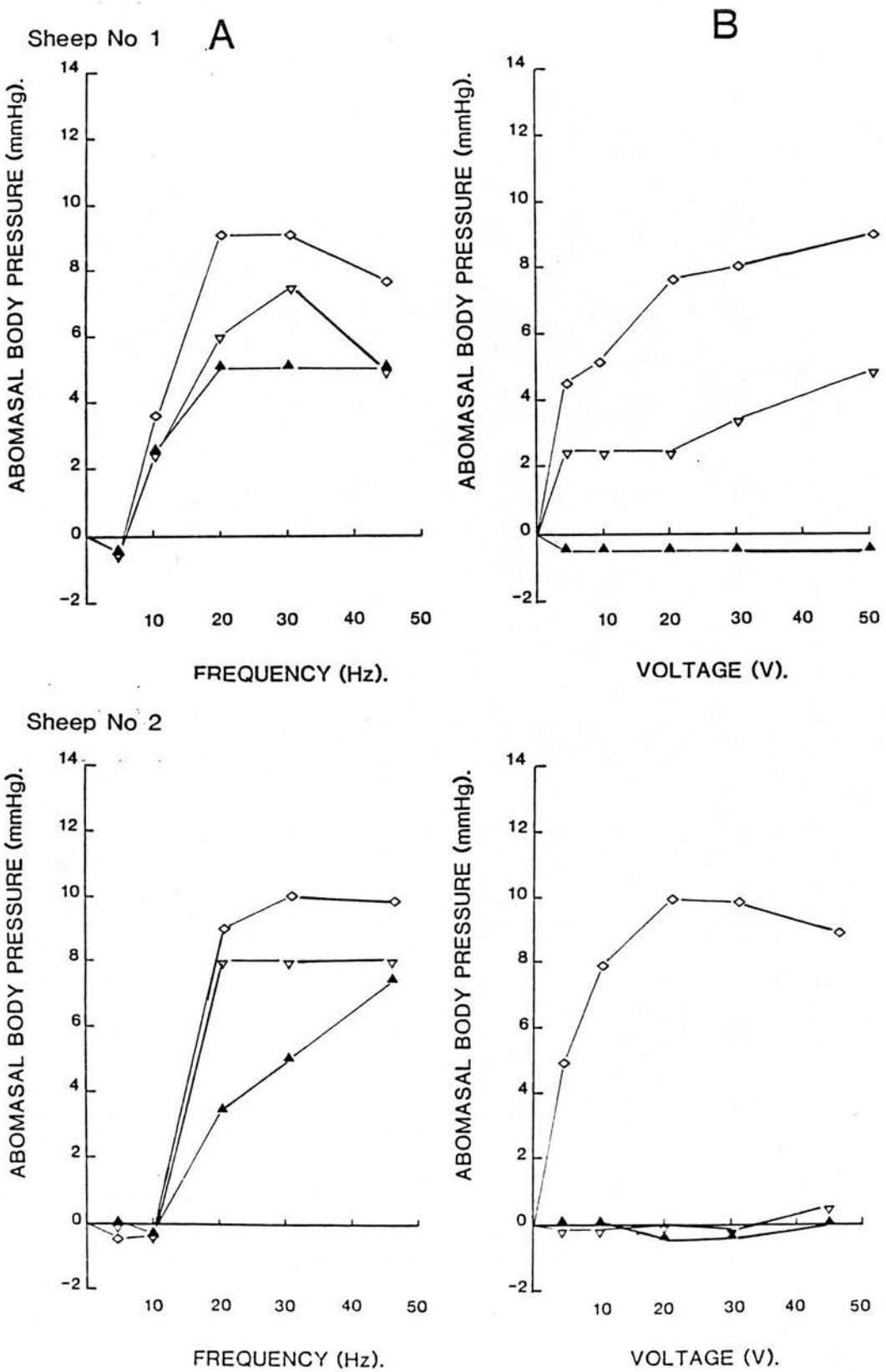
Electrical excitation of the peripheral end of the cut cervical vagus therefore demonstrates that the vagus exerts an excitatory and inhibitory influence on the motor profile of both the abomasal body and antrum. The manifestation of the inhibitory control would seem to fit the proposed function of each region: relaxation of the body is appropriate to its function as a food store; the capacity of vagal reduction of the amplitude of antral contraction provides an efferent mechanism whereby, for example, the distal intestinal tract may regulate the rate of abomasal emptying. This author is unwilling to form firm conclusions on the basis of the results of electrical stimulation of nerves.

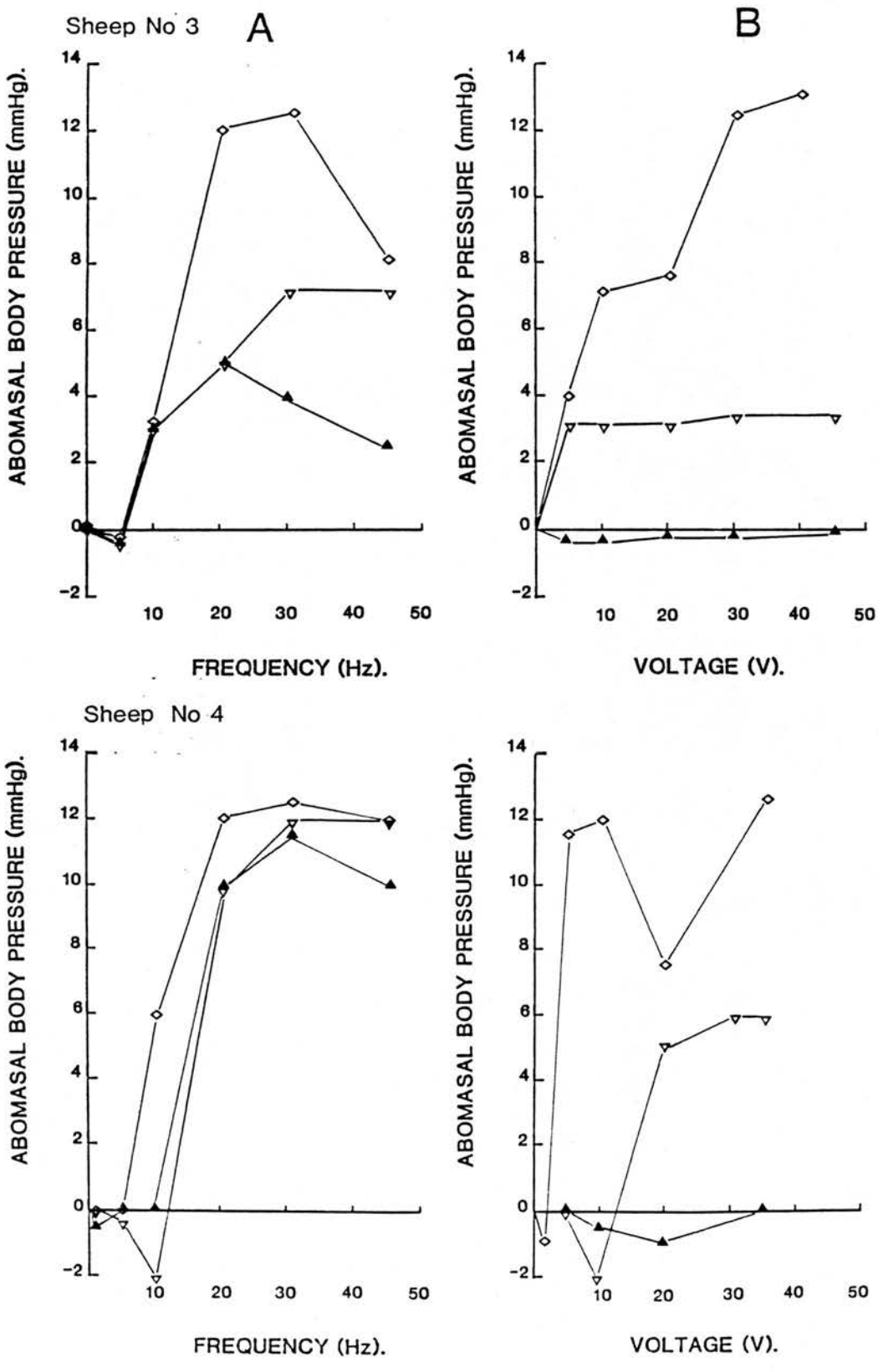
Figure 5.

Frequency-response curves (A) and voltage-response curves (B) showing the effect of electrical stimulation of the peripheral end of the cut cervical vagus on the abomasal body. Low frequency and voltage combinations tended to cause a relaxation of the body; higher frequency and voltage combinations caused contraction of the abomasal body.

Key.

Frequency Response Curves.		Symbol.	Voltage Response Curves.		
5 Hz.	=	Filled Triangles (▲).	=	5 V.
10 Hz	=	Open Triangles (▽).	=	10 V.
30/35 Hz	=	Open Diamonds (◇).	=	10 V.


Sheep No 1
A
B
ABOMASAL BODY PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).
Sheep No 2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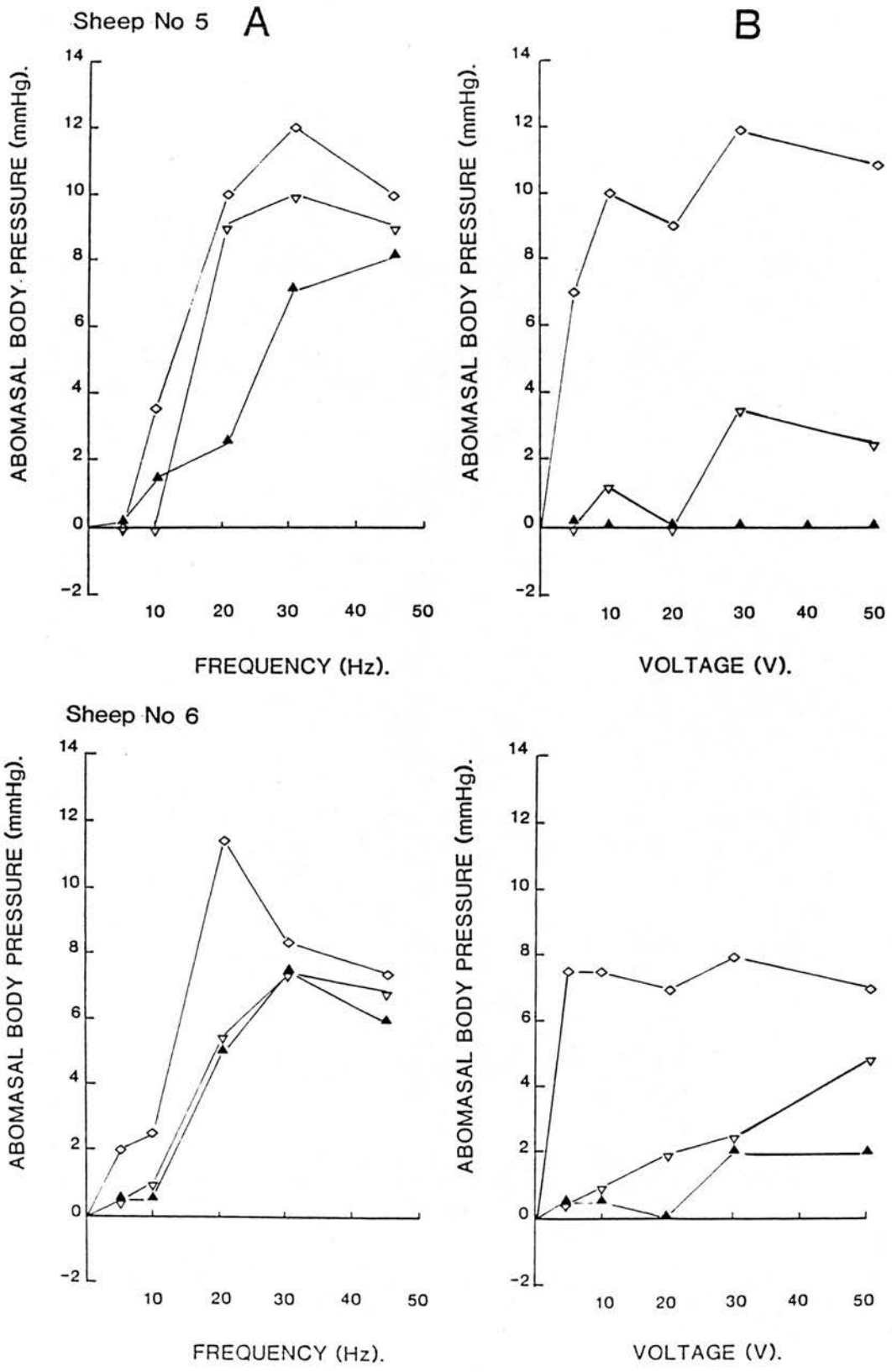
A
B
ABOMASAL BODY PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).
Sheep No 4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A
B
ABOMASAL BODY PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).
Sheep No 6

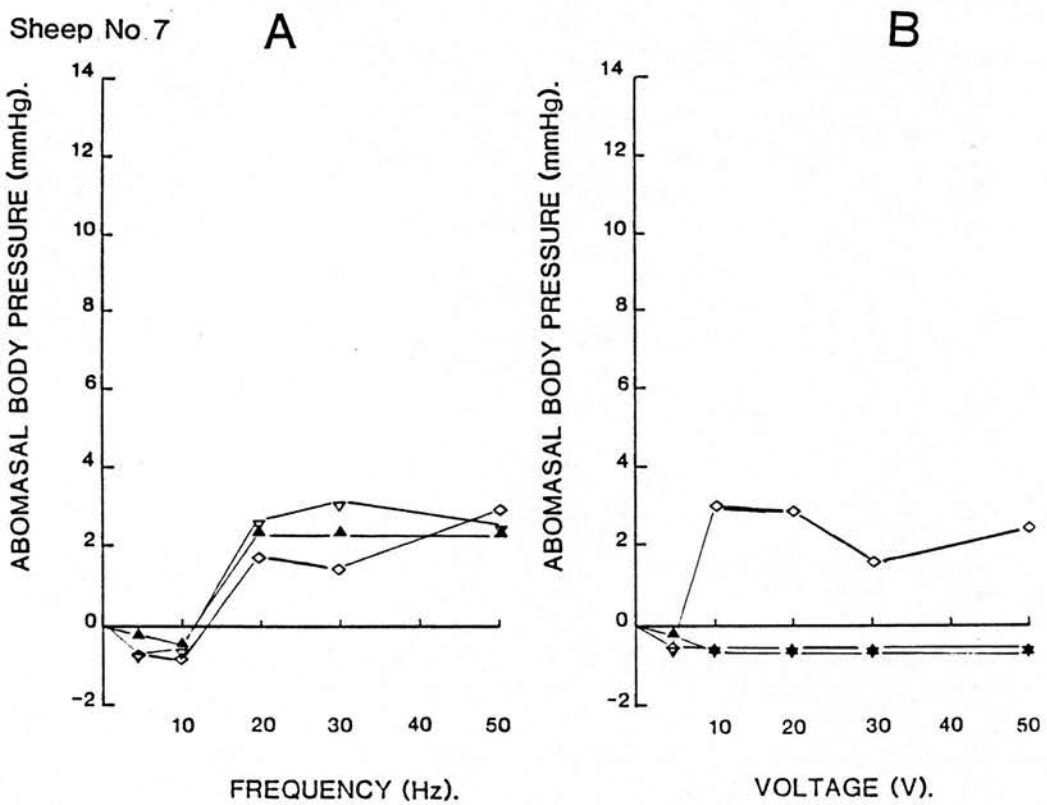
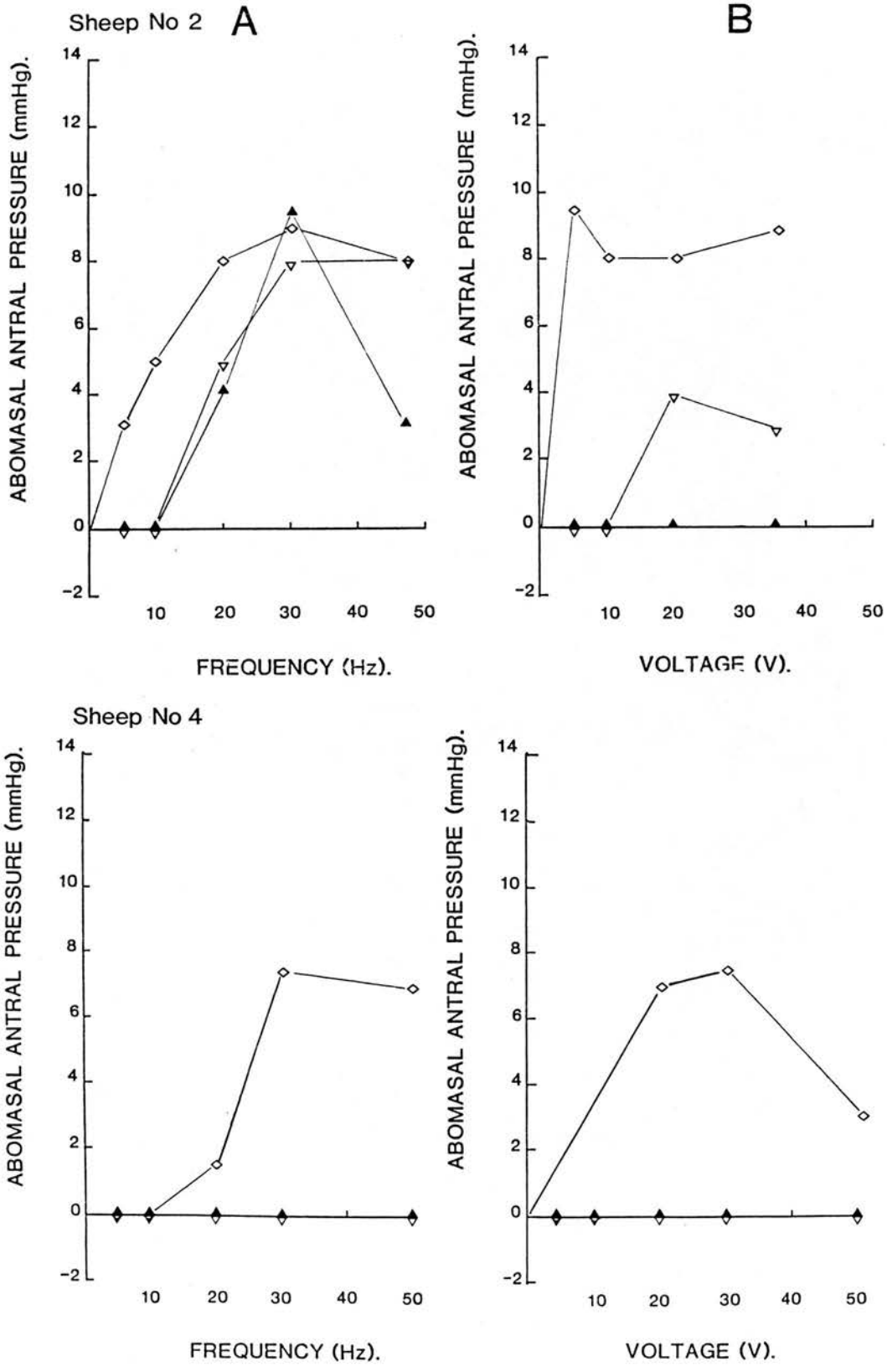
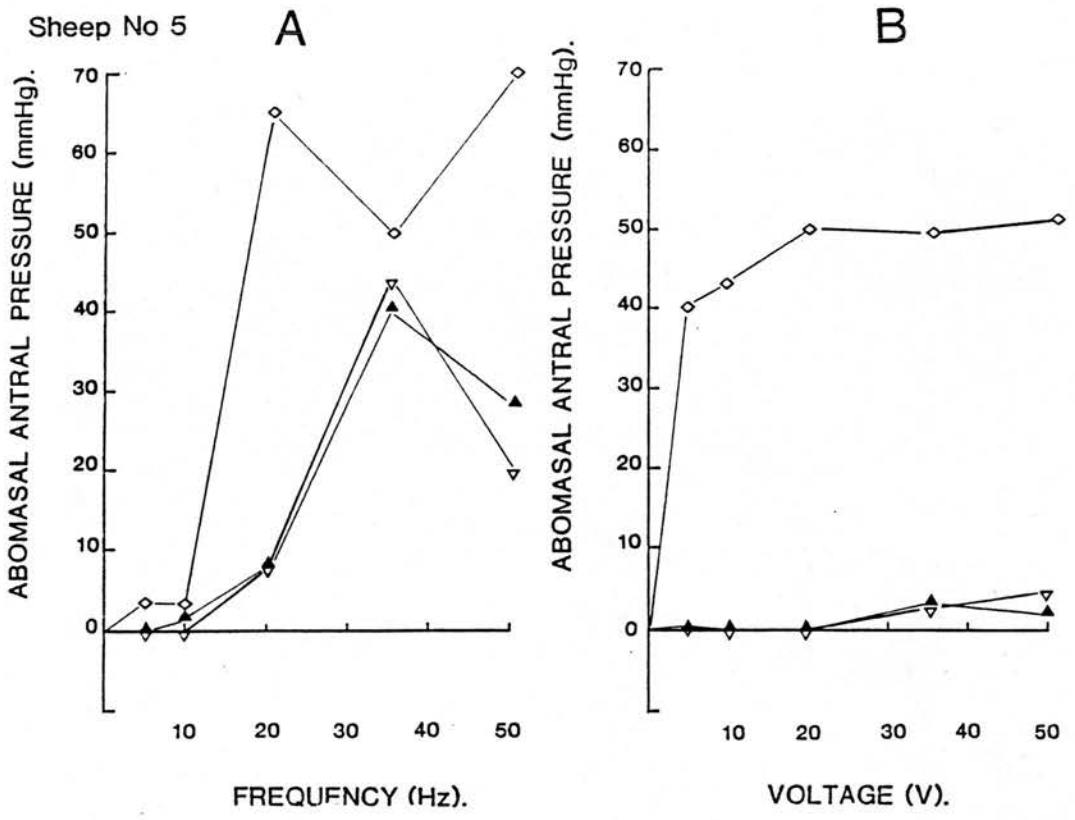

Sheep No. 7
A
B
ABOMASAL BODY PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).

Figure 6.

Frequency-response (A) and voltage-response (B) curves showing the contractile response of the abomasal antrum to electrical stimulation of the peripheral end of the cut cervical vagus.

The key to the symbols is the same as that used for figure 5.


Sheep No 2
A
B
ABOMASAL ANTRAL PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).
Sheep No 4


Sheep No 5
A
B
ABOMASAL ANTRAL PRESSURE (mmHg).
70
60
50
40
30
20
10
0
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).

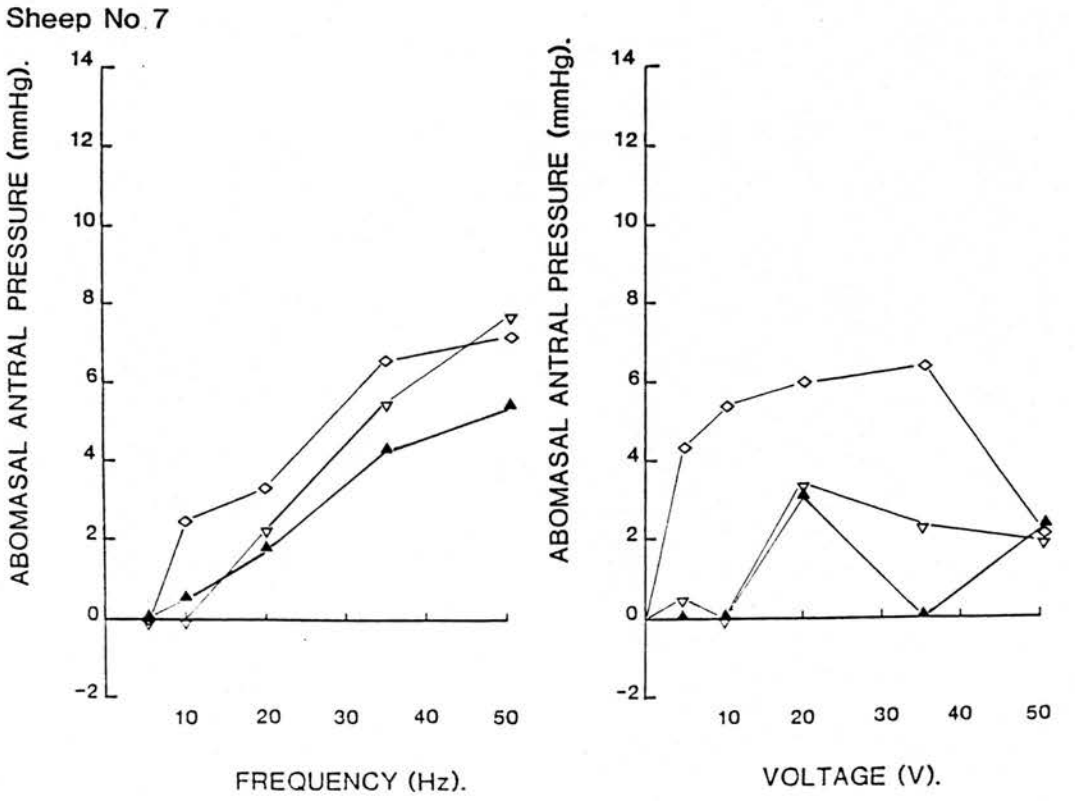

Sheep No 7
ABOMASAL ANTRAL PRESSURE (mmHg).
14
12
10
8
6
4
2
0
-2
10
20
30
40
50
FREQUENCY (Hz).
VOLTAGE (V).

Figure 7.

The effect on abomasal body pressure of low frequency (5-10 Hz) electrical stimulation of the peripheral end of the cut cervical vagus at various voltages. In each of A, B and C the period of electrical stimulation is shown by the horizontal bar; stimulus parameters are shown above the bar.

In 'A' body relaxation occurs during the period of electrical stimulation.

In 'B' the body shows no response during electrical stimulation but contracts immediately electrical stimulation stops. Such 'off-contractions' are thought to be indicative of electrical activation of vagal non-adrenergic, non-cholinergic fibres.

In 'C' the body relaxes during electrical stimulation and contracts immediately electrical stimulation stops.

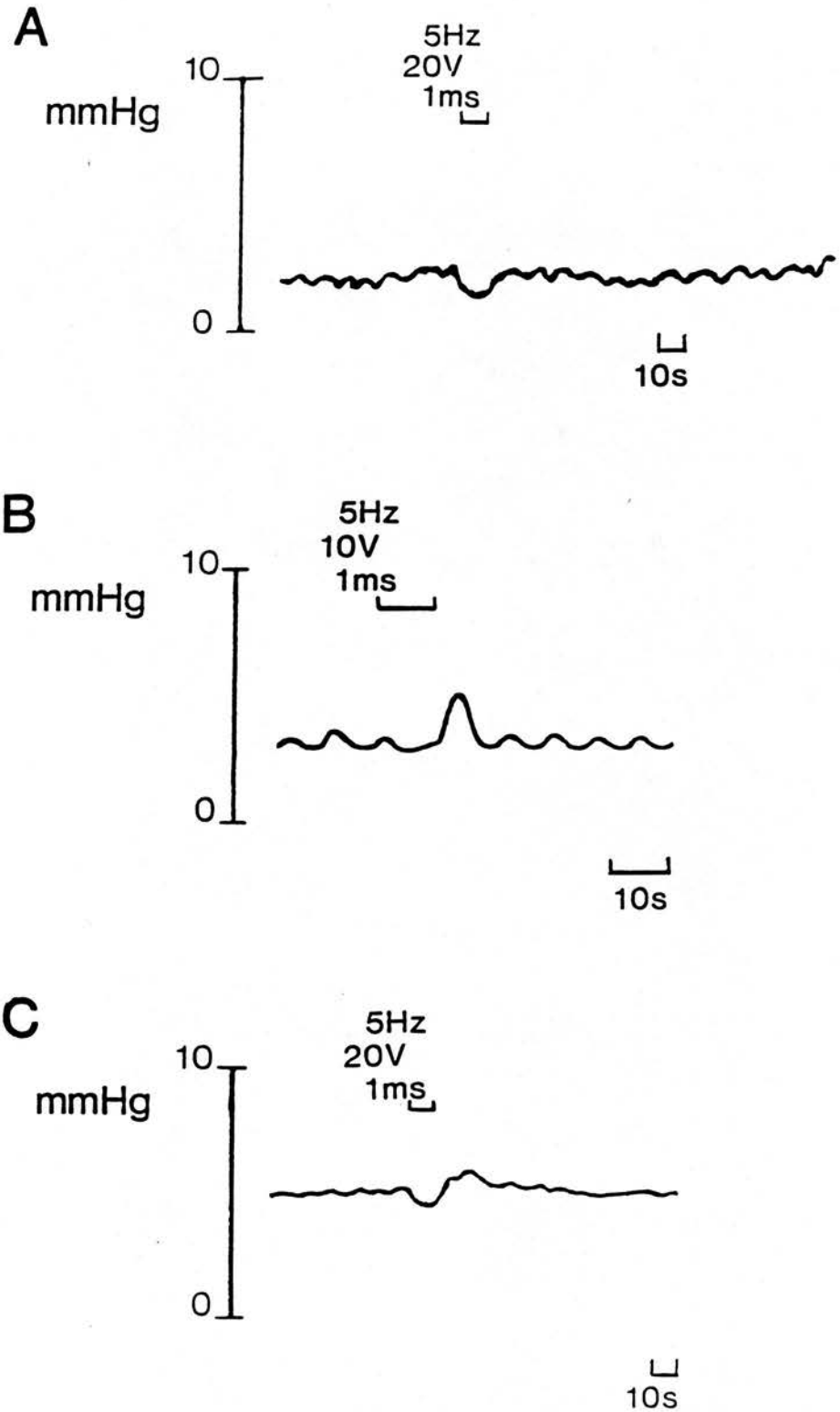

A
5Hz
20V
1ms
10
mmHg
0
10s
B
5Hz
10V
1ms
10
mmHg
0
10s
C
5Hz
20V
1ms
10
mmHg
0
10s

Figure 8.

The effect on abomasal body pressure of higher (10-50 Hz) frequency electrical stimulation of the peripheral end of the cut cervical vagus at different voltages. In each of A, B, C and D the period of stimulation is denoted by the horizontal bar; stimulus parameters are shown above the bar.

In 'A' electrical stimulation of the peripheral end of the cut cervical vagus caused a biphasic response; the body first relaxes transiently ('pre-contraction relaxation') and then contracts. The contraction stops soon after electrical stimulation stops and pressure falls directly to a steady level. B, C and D illustrate other ways in which similarily evoked body contractions were seen to end.

B:- a post-contraction relaxation occurs.

C:- body pressure falls to a steady level in two phases, a fast first phase (2.5 s) and a slow second phase (20 s).

D:- body pressure oscillates before stabilizing.

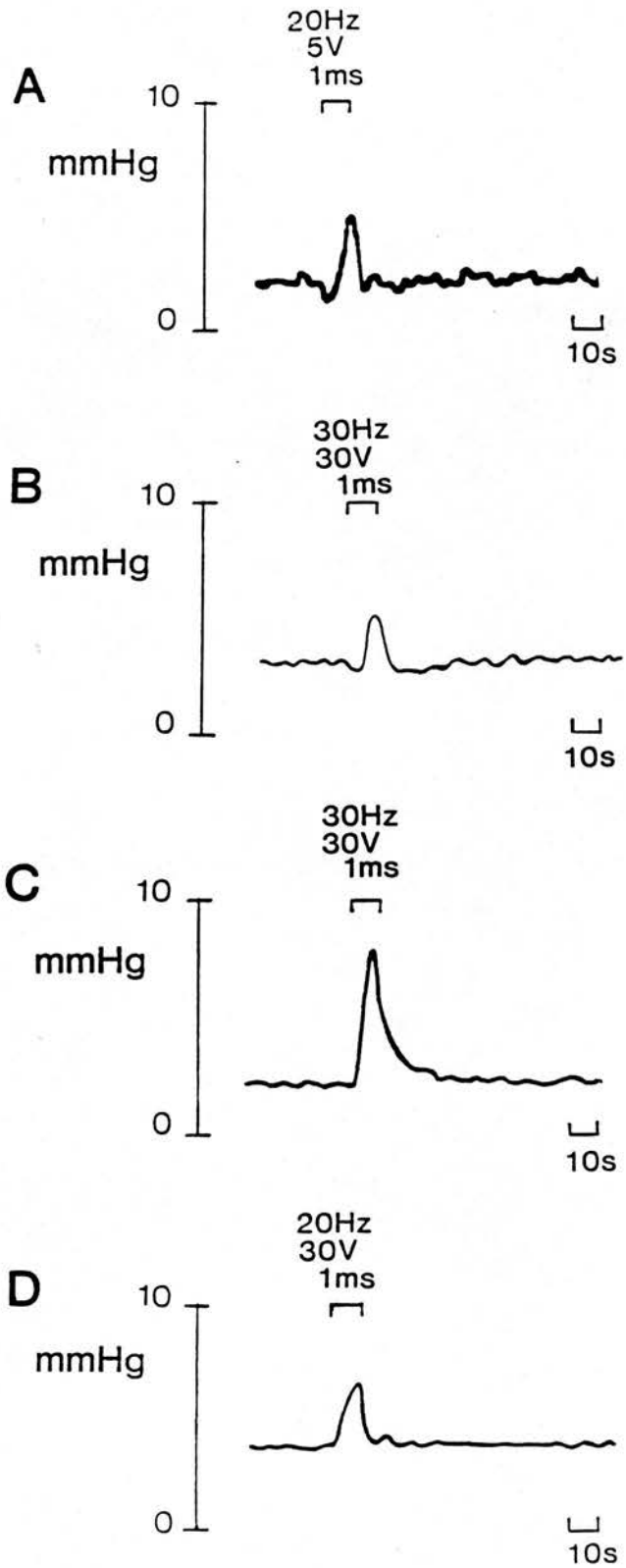

A
20Hz
5V
1ms
10
mmHg
0
10s
B
30Hz
30V
1ms
10
mmHg
0
10s
C
30Hz
30V
1ms
10
mmHg
0
10s
D
20Hz
30V
1ms
10
mmHg
0
10s

Figure 9.

The inhibition of antral contractions elicited by electrical stimulation of the peripheral end of the cut cervical vagus. Antral contraction amplitude falls during the period of electrical stimulation (denoted by the horizontal bar). Stimulus parameters are given above the bar. Post-stimulus contractions were often of larger amplitude than control contractions.

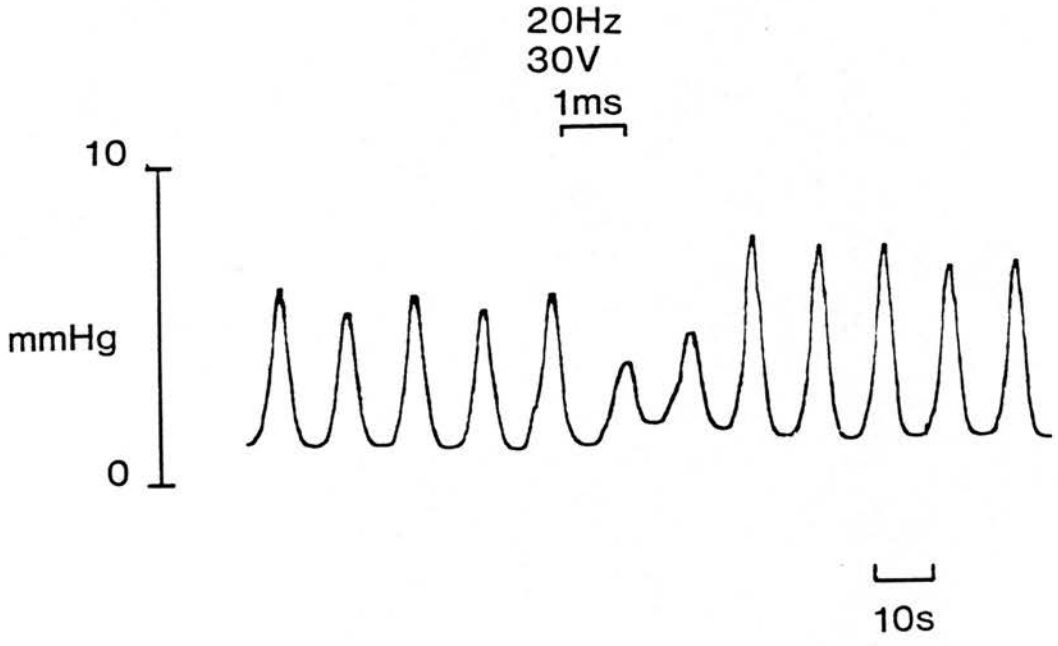

20Hz
30V
1ms
10
mmHg
0
10s

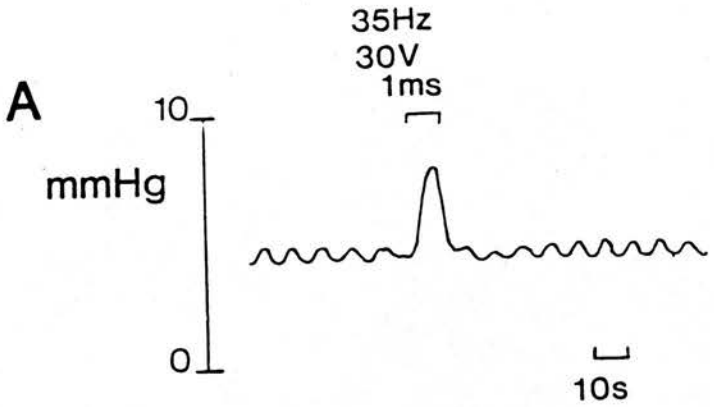
Figure 10.

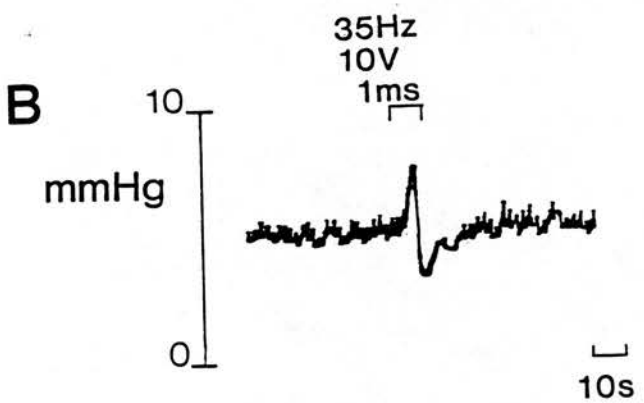
The range of abomasal antral excitatory responses to electrical stimulation of the peripheral end of the cut cervical vagus. Electrical stimulation (denoted by the horizontal bar in each of A, B and C) evoked a contraction which ended soon after the period of electrical stimulation. Stimulus parameters are given beneath the bar in each case.

A:- antral pressure returns directly to a steady level.

B:- a post-contraction relaxation occurs.

C:- post-contraction pressure oscillates before stabilizing.


35Hz
30V
1ms
A
10
mmHg
0
10s


35Hz
10V
1ms
B
10
mmHg
0
10s


45Hz
20V
1ms
C
10
mmHg
0
10s

CHAPTER FIVE.

THE EFFECT OF ABOMASAL BODY INFLATION ON THE AMPLITUDE OF ANTRAL CONTRACTIONS.

INTRODUCTION.

Gastro-gastric reflexes are involved in the control of stomach motility in monogastric animals. Stomach distension is implicated in regulation of stomach emptying in man (Hunt and McDonald, 1954; Erskine and Hunt, 1981; Bateman, 1982), dogs (Strunz and Grossman, 1978) and ferret (Andrews, Grundy and Scratcherd, 1980a; Andrews and Scratcherd, 1980); abomasal distension has been implicated in the regulation of abomasal emptying in pre-ruminant calves (Bell and Watson, 1976; Bell, Holbrooke and Titchen, 1977). Studies of alimentary reflexes in mature ruminants have concentrated on the forestomach (Leek and Harding, 1974) although an abomaso-reticulum reflex has been described (Titchen, 1958; Iggo and Leek, 1967b). In the transected stomach preparation of urethane-anaesthetized ferrets inflation of the body pouch increases the amplitude of antral pouch contractions in a vagally-dependent manner (Andrews, Grundy and Scratcherd, 1980a). Little information is available about the existence of possible abomaso-abomasal reflexes that may regulate abomasal motility in adult sheep. Experiments were designed to look for an body-antral reflex in the ovine abomasum, corresponding to the body-antral reflex identified in the ferret stomach (Andrews, Grundy and Scratcherd, 1980a)

PROTOCOL.

The effects on the amplitude of antral contraction of inflation of a balloon in the body were assessed in six types of abomasal preparation:-

1. Fully innervated, intact abomasum.
2. Extrinsically denervated, intact abomasum.
3. Fully extrinsically innervated, transected abomasum.
4. Splanchnotomized transected abomasum.
5. Vagotomized, transected abomasum.
6. Extrinsically denervated, transected abomasum.

Consideration was also given to the effect of inflation of the abomasal body balloon on the abomasal body.

RESULTS.

1. Fully innervated, intact abomasum.

A balloon was inflated with 100-1000 ml of air in the body of a fully innervated intact abomasum on a total of 16 occasions in 5 sheep. The peak-to-trough amplitude of the 10 antral contractions preceding and following each body inflation was measured for analysis. Each inflation produced significant increases in antral contraction amplitude (mean increase +/- s.d. = 2.4 +/- 2.1 mmHg, mean percentage increase +/- s.d. = 320.0 +/- 172.1 %) (fig. 11). The frequency of antral contraction was not affected by body inflation. Omental manipulation did not alter the rate or amplitude of antral contraction.

2. Extrinsically denervated, intact abomasum.

In one animal the abomasal body was inflated to 1000 ml twice before and twice after section of the abdominal continuations of the dorsal and ventral vagi proximal to the omaso-abomasal junction. Prior to neurotomy, inflation of the body caused a mean increase in antral contraction amplitude of 6.3 mmHg, s.d. = 0.8 mmHg (mean percentage increase +/- s.d. = 586.5 +/- 156.2 %). Subsequent to neurotomy the evoked increase in antral contraction amplitude, although present, was much reduced (mean increase +/- s.d. = 0.9 +/- 0.3 mmHg, mean percentage increase +/- s.d. = 143.4 +/- 16.8 %).

3. Extrinsically innervated, transected abomasum.

The suitability of the transected abomasal preparation for the determination of abomasal reflex mechanisms is discussed in Chapter 3.

Balloon inflation with 200-900 ml of air in the abomasal body pouch of an extrinsically innervated, transected abomasum significantly increased antral contraction amplitude (mean increase +/- s.d. = 0.9 +/- 0.6 mmHg, mean percentage increase +/- s.d. = 223.1 +/- 184.9 %) on 28 out of 29 trials in 11 sheep (fig. 11, 12).

4. Splanchnotomized, transected abomasum.

In one sheep the abomasal body balloon was inflated with suprathreshold volumes of air (500-900 ml) before (n = 3) and after (n = 5) section of the splanchnic nerves at the

level of the crus of the diaphragm. Significant increase in antral contraction occurred with each inflation before and after splanchotomy. There was no significant difference in the mean increase (mean percentage increase) in antral contraction amplitude elicited by body inflation before and after splanchotomy, which were 1.85 +/- 0.27 mmHg (197.0 +/- 2.1 %) and 1.42 +/- 0.23 mmHg (220.6 +/- 33.8 %) respectively. Splanchnic section reduced from 500 ml to 200 ml the threshold volume of abomasal body inflation required to increase antral contraction amplitude (fig. 12).

5.Vagotomized, transected abomasum.

In four sheep the effects on antral pouch contraction amplitude of inflation of the body pouch balloon before and after cervical vagotomy were compared. In three sheep, body inflation produced a significant increase in antral contraction amplitude prior to vagotomy but no significant increase in antral contraction amplitude after vagotomy. In the fourth sheep, significant increases in antral contraction amplitude occurred with body inflation before and after cervical vagotomy, but the volume of body distension required to elicit an increase in antral contraction amplitude was 700 ml post-vagotomy as opposed to 200 ml pre-vagotomy. Section of the abdominal continuations of the dorsal and ventral vagi abolished the antral response to body inflation in this preparation.

6.Extrinsically denervated, transected abomasum.

Inflation of the body pouch balloon produced no effect on

the amplitude of antral pouch contractions in preparations where the abdominal continuations of the dorsal and ventral vagi had been sectioned (n = 4).

An increase in the amplitude of the first antral contraction to occur after body balloon inflation was observed in all preparations. Body balloon deflation usually produced an rapid decrease in the amplitude of antral contraction (fig. 11, 12) although the amplitude of antral contraction could remain elevated for up to five minutes post-deflation.

Unless conditions were changed by neurotomy or abomasal transection the minimum ('threshold') volume of body inflation required to elicit an increase in antral contraction amplitude remained constant for all preparations. The range of threshold volumes and pressures (100-1000 ml, 5.5-15.0 mmHg) were similar for both transected and untransected preparations.

Ramp (inflation in 100 ml increments with a minimum of 30 s between increments) and step (inflation in 100 ml increments as quickly as possible, usually within 15 s) inflations of the abomasal body were carried out. Step inflation produced a 'peak and plateau' pattern in the record of abomasal body pressure but the 'peak' was small and the 'plateau' phase was achieved in less than 1 min (fig. 11, 12). The body pressure achieved 1 min after inflation depended on the volume of inflation and was the same for ramp and step inflations (fig. 13). Inflations produced similar increases in body pressure before and

after denervation (fig. 12). The threshold inflation required to elicit an increase in antral contraction amplitude was the same for ramp and step inflations.

Although in the transected abomasal preparations the body and antral pouches were physically separated as much as possible, in some experiments body inflation produced an apparent increase in antral tone (fig. 12). Because the magnitude of the apparent increase in antral tone produced by inflation of the body balloon was not affected by neurotomy and followed exactly the time course of body balloon inflation (fig. 12), the apparent 'tone' increase was likely to be due to body balloon inflation pushing the wall of the body pouch against the wall of the antral pouch. Thus it was an increase in antral pressure rather than an increase in antral tone. It was interesting to note that the increase in antral pressure produced by body inflation did not elicit an intrinsic antro-antral excitatory reflex (fig. 12) as described in the rabbit (Deloof and Rousseau, 1985). Increase in antral pressure was not necessary for the increase in antral contraction amplitude induced by body inflation (fig. 11).

In 4 transected abomasal preparations, body distension did not elicit an increase in antral contraction amplitude.

DISCUSSION.

The results demonstrate that acute abomasal body distension elicits a reflex increase in antral contraction amplitude in the majority of preparations. The apparent

absence of the reflex from 4 preparations may be the result of inhibitory mechanisms activated by the extensive surgical preliminaries necessary in these experiments (Abrahamsson, Glise and Glise 1979). The rate of onset and offset of the response to the stimulus indicates that the mechanism whereby antral contraction amplitude is increased by body inflation is neurally mediated. The lack of effect of stretching the lesser omentum indicates that omental receptors are not involved. The persistence of the reflex after splanchotomy and the abolition of the reflex by vagotomy in the transected preparation confirms that the reflex has a vago-vagal component. Evidence exists for a sensory neural apparatus capable of eliciting such a reflex. The presence in the abomasal wall of distension-sensitive receptors is implied by the studies of abomasal reflexes by Phillipson (1939) and Titchen (1958). Single unit recordings at the cervical and central level have identified distension-sensitive mechanoreceptors in both the stomach of monogastric animals (Paintal, 1953, 1954; Iggo, 1955; Clarke and Davison, 1974; Andrews, Grundy and Scratcherd, 1979; Jeanningros 1984) and in the abomasum of ruminants (Harding and Leek 1972b, 1973; Cottrell, D.F. and Reynolds, G.W., personal communication).

Andrews, Grundy and Scratcherd (1980a) maintain that both the afferent and efferent limbs of the proposed body-antrum reflex of the ferret stomach are in the vagus. The small increase in antral contraction amplitude brought about by body distension in the sheep after denervation of the intact abomasum may be due to an intramural reflex. This

reflex is possibly mediated by vagal cholinergic post-ganglionic fibres such as were suggested for the local antro-antral reflex described in the rabbit (Deloof and Rousseau, 1985) and ferret (Grundy, Hutson and Scratcherd, 1986). It may be due to a decrease in the activity of intramural non-adrenergic, non-cholinergic inhibitory fibres. The results of abomasal transection imply that the abomasal body exerts an intramural inhibitory influence on antral contraction amplitude (Chapter 3). The possibility of the results being an artefact caused by body inflation changing the recording conditions in the antrum is unlikely, as body inflation could produce marked effects on antral contraction amplitude while producing little effect on antral tone (fig. 11).

The persistence of the reflex in one cervically-vagotomized transected abomasal preparation until section of the abdominal continuations of the dorsal and ventral vagi suggests the existence of a reflex loop possibly involving the coeliaco-mesenteric ganglia. Alimentary reflexes mediated through decentralized mesenteric ganglia have been described in the cat (Kuntz and Saccomanno, 1944), dog (Schapiro and Woodward, 1959) and guinea-pig (Kruelen and Szurszewski, 1979). Abomasal distension up to severe levels failed to elicit a similar response in three similar preparations, so the importance of such a reflex loop in sheep involving the coeliaco-mesenteric ganglia remains unresolved.

The effect of body inflation on antral contraction

amplitude and antral pressure were independent of each other. This suggests that the increase in antral contraction amplitude was a result of a body-antral reflex rather than an indirect result stemming from an induced increase in antral pressure.

The functional significance of reflexes demonstrated in acutely prepared animals is difficult to assess. As increases in antral contraction amplitude may be brought about by small increases in body pressure, the body-antral reflex must be more than a 'safety valve' mechanism for maintaining abomasal pressure within certain limits. If the reflex is a mechanism for increasing the rate of abomasal emptying it is likely to be subject to constraints imposed by the state of digestion in both the abomasum and the digestive tract distal to the abomasum. If body distension increases the rate of abomasal emptying, mechanisms may exist to promote the activity of the digestive tract distal to the abomasum to allow for the increase in digesta flow rate.

Andrews, Grundy and Lawes (1980) found that step inflation of the body of the ferret stomach causes an initial inhibition of antral contraction not seen with ramp inflation. They proposed that step increases in stomach volume do not occur 'in nature', and so ramp inflation of the stomach is a more physiological stimulus than step inflation. This may not be a valid criticism as it is likely that drinking of large volumes of fluid and food ingestion in carnivores does result in step increases in stomach volume in monogastric animals. It is possible that the inhibition of antral contraction

amplitude induced by step increase in volume of the ferret stomach during the period of accommodation of the body is a mechanism to prevent stomach pressure reaching excessive levels rather than a result of stomach pressure having reached excessive levels. No period of inhibition of abomasal antral contraction was seen with step inflation of the abomasal body. As step increases in abomasal pressure are unlikely in adult ruminants because of the volume buffering capacity of the forestomach such a mechanism may be unnecessary.

Step inflations produced a 'peak and plateau' pressure pattern in the body indicating that the body relaxes in response to an increase in internal pressure. Electrical stimulation of the peripheral end of the cut cervical vagus suggests that the abomasal body has an inhibitory vagal innervation (Chapter 4). However, step inflations of the body produced similar increases in body pressure before and after vagotomy. Therefore the relaxatory capacity of the body must be mediated through the intrinsic nervous system and/or be a property inherent to the smooth muscle of the body. This is in contrast to the vago-vagal body-body relaxatory reflex described for the cat (Abrahamsson, 1973a) and ferret (Andrew, Grundy and Lawes, 1980a, 1980b; Andrews and Scratcherd, 1980; Andrews and Lawes, 1982). The smooth muscle of the monogastric stomach has properties conducive to its volume **accommodating** role :-

a). Extrinsically denervated strips of smooth muscle from the body of the monogastric stomach develop less tension

in response to stretch than a similar muscle strip derived from the antrum (Szursweski, 1981). This means that body muscle will stretch more in response to a given pressure than antral muscle.

b). The resting membrane potential of the smooth muscle of the body and fundus of the monogastric stomach is relatively low (Szursweski, 1981). This means that at their resting potential, body and fundus smooth muscle cells are partly contracted, and will therefore relax in response to hyperpolarizing influences.

It is not known if the intrinsically innervated smooth muscle of the abomasal body has the inherent properties of low resting membrane potential and high elasticity displayed by the intrinsically innervated smooth muscle of the body and fundus of the monogastric stomach.

The body of the abomasum appears to be less well adapted for limiting pressure changes in response to distension than the body and fundus of the monogastric stomach.

a). Distension produces a 'peak and plateau' in the pressure of the abomasal body. However the 'peak' pressure: 'plateau' pressure ratio for the abomasal body was much less than the 'peak' pressure: 'plateau' pressure ratio of the body and fundus of the ferret stomach (Andrews, Grundy and Lawes, 1980, fig. 2).

b). In lacking a true fundus the abomasum (Dyce, Sack and Wensing, 1987) may be less well adapted anatomically than the monogastric stomach to minimize the pressure effects of

distension.

c). Receptive relaxation of the body and fundus of the monogastric stomach can be mediated through the extrinsic innervation by mechanical stimulation of the oesophagus or pharynx (Abrahamsson and Jansson, 1969) or the stomach (Abrahamsson, 1973a, 1973b; Andrews, Grundy and Lawes, 1980). No evidence was found in the chloralose-anaesthetized sheep for equivalent extrinsically mediated functional reflexes arising in either the reticulum (Chapter 6) or the abomasal body eliciting relaxation of the abomasal body.

The function of the body and fundus of the monogastric stomach in some species is to act as a distensible receptacle for food to allow the animal to eat large meals at long intervals. To this end it is provided with an array of mechanisms whereby large meals may be accommodated with little change in stomach pressure. The feeding behaviour of ruminants and the volume buffering effect of the forestomachs mean that the abomasum is not subjected to large meals at long intervals. This may explain why the abomasal body has a relatively small capability to accommodated changes in volume with little change in body pressure.

Figure 11.

A. The excitatory effect of inflation of the abomasal body on the motility of the antrum of a fully innervated intact abomasum. The periods of body inflation are shown by the horizontal bars; inflation volume is given above the bar. The upper trace shows abomasal antrum pressure; the lower trace shows the abomasal antrum integrated e.m.g.

Note:-

a). The lack of effect of body inflation on antral tone.

b). The rapid fall in antral contraction amplitude on deflation of the body.

B. The excitatory effect of inflation of the abomasal body on the motility of the antrum of an extrinsically denervated surgically transected abomasum. Physical contact between the body and antrum was prevented as far as possible. The upper trace shows abomasal body volume; the middle trace shows abomasal antrum pressure and the lower trace shows abomasal antrum integrated e.m.g.

Note the 'peak and plateau' pressure effect produced by inflation of the abomasal body pouch.

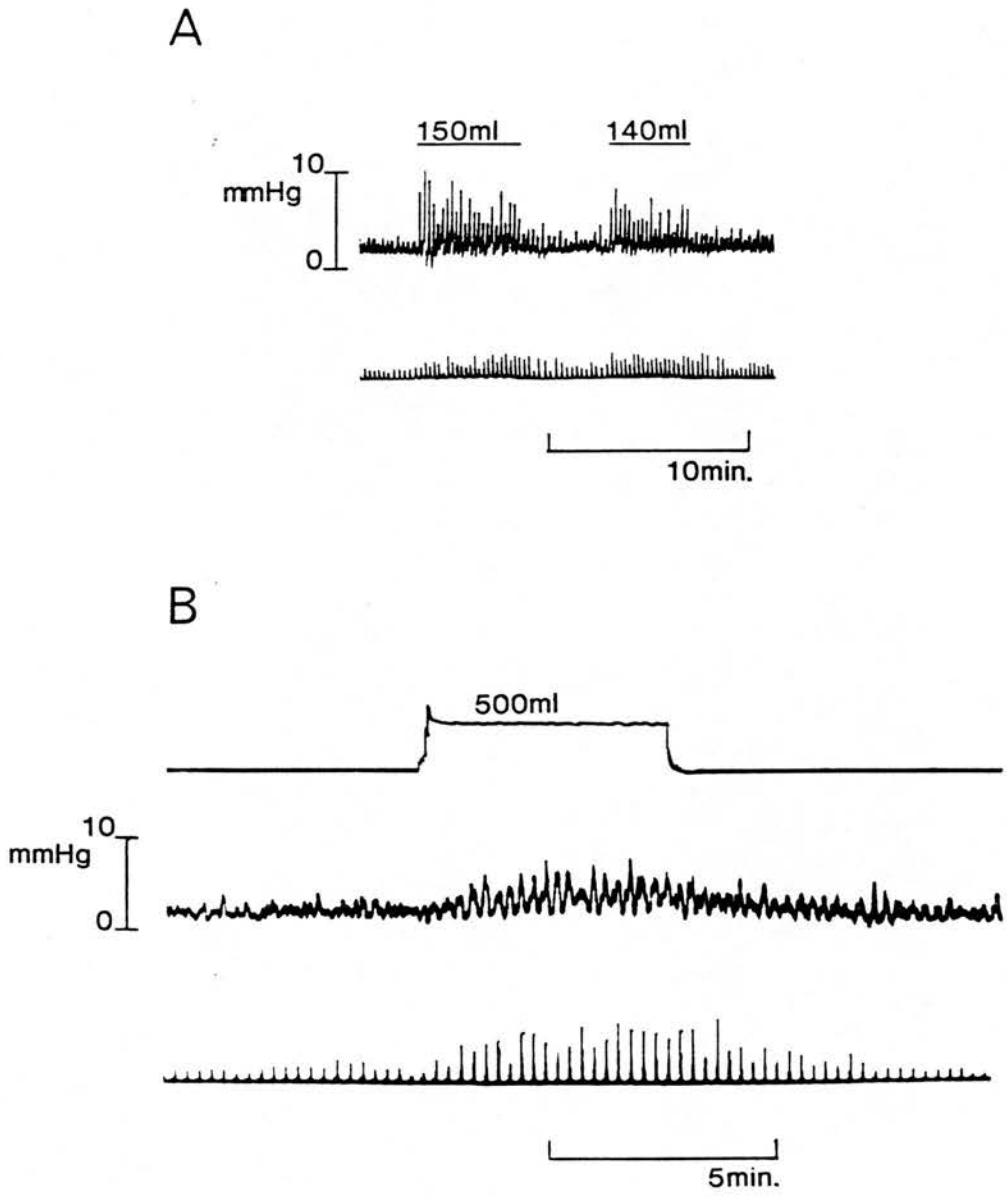

A
150ml
140ml
10
mmHg
0
10min.
B
500ml
10
mmHg
0
5min.

Figure 12.

The effect of inflation in 100 ml increments of the abomasal body (upper trace) on the abomasal antrum pressure (middle trace) and abomasal integrated e.m.g. (lower trace) (A) prior to neurotomy (B) subsequent to splanchnotomy and (C) subsequent to total extrinsic denervation by section of the abdominal continuations of the dorsal and ventral vagi at the level of the omaso-abomasal junction. The threshold body inflation required to elicit increase in antral activity is best seen on the e.m.g. trace in each case. Prior to neurotomy the threshold is 400-500 ml; subsequent to splanchnotomy the threshold is 100-200 ml; subsequent to denervation body inflation does not affect antral motility.

Note:-

a). That the magnitude of the tone increase in the abomasal body produced by inflation with volumes of up to 1500 ml was not affected by neurotomy.

b). Even after total neurotomy body inflation produced an apparent increase in antral tone. This was due to body inflation pushing the wall of the body pouch against the wall of the antral pouch.

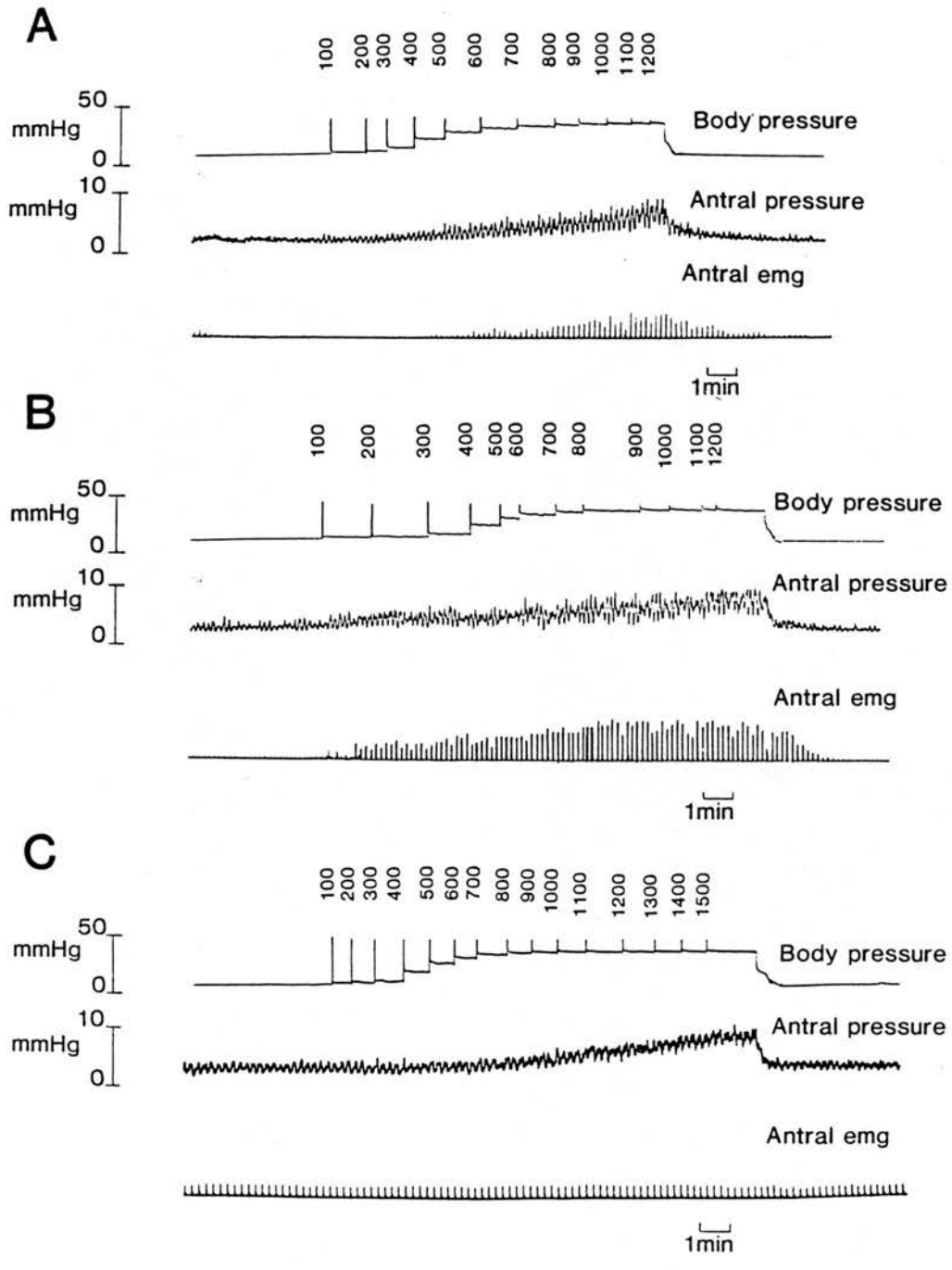
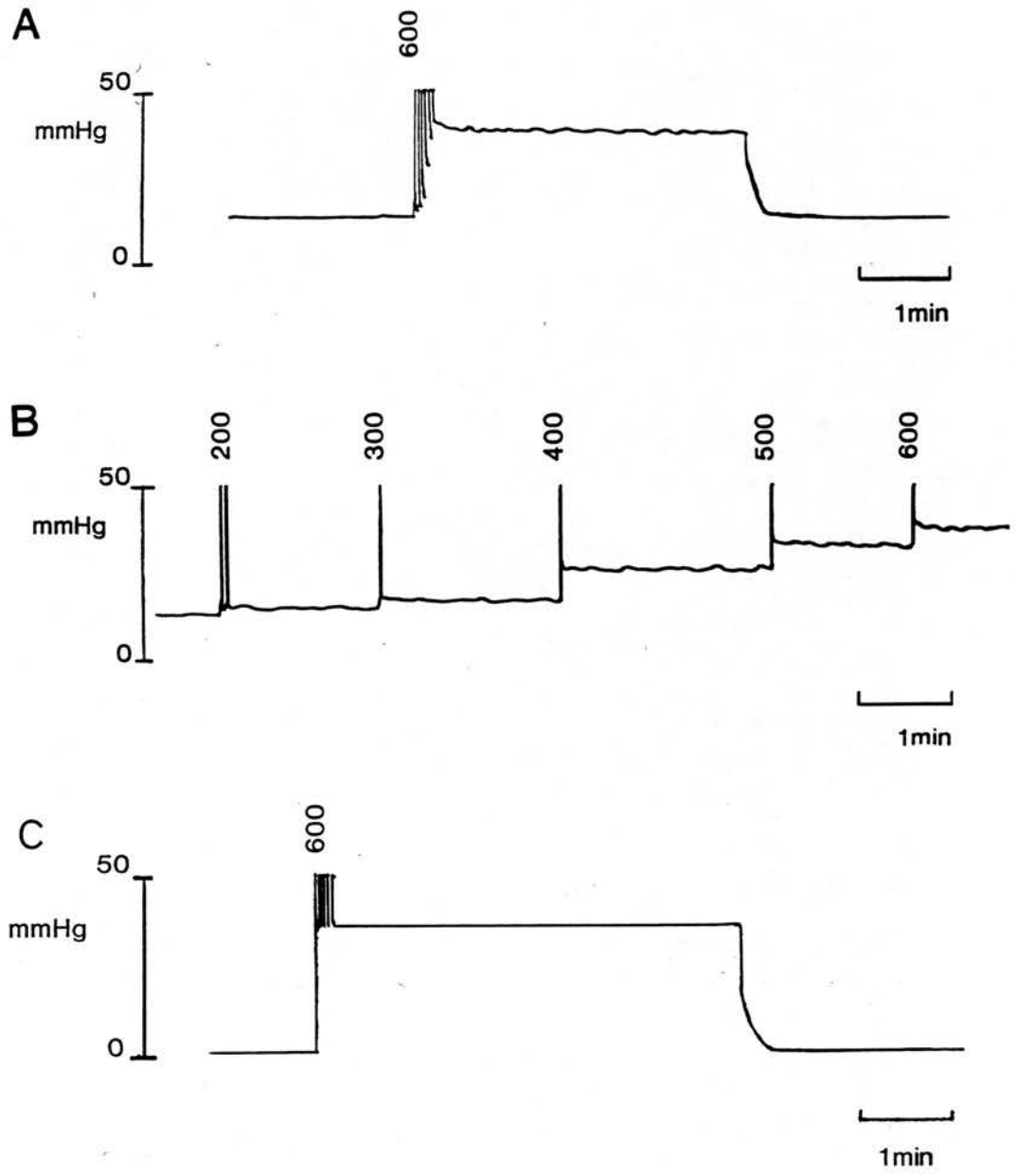

A
100
200
300
400
500
600
700
800
900
1000
1100
1200
50
mmHg
0
Body pressure
10
mmHg
0
Antral pressure
Antral emg
1min
B
100
200
300
400
500
600
700
800
900
1000
1100
1200
50
mmHg
0
Body pressure
10
mmHg
0
Antral pressure
Antral emg
1min
C
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
50
mmHg
0
Body pressure
10
mmHg
0
Antral pressure
Antral emg
1min

Figure 13.

Comparison of the effects on abomasal body pressure of a step inflation to 600 ml (A) and a ramp inflation in 100 ml increments over 7.5 minutes to 600 ml (B) shows that the body pressure achieved in both instances was the same.

Note the 'peak and plateau' shape produced in the abomasal body pressure trace by the step inflation. For comparison the pressure events produced by step inflation of an isolated balloon are shown (C).


A
600
50
mmHg
0
1min
B
200
300
400
500
600
50
mmHg
0
1min
C
600
50
mmHg
0
1min

CHAPTER SIX.

THE EFFECT OF RETICULAR INFLATION ON ABOMASAL BODY TONE.

INTRODUCTION.

Electrical stimulation of the peripheral end of the cut cervical vagus has been shown to induce relaxation of the abomasal body, indicating that the abomasal body is innervated by vagal inhibitory fibres (Chapter 4). The functional significance of this proposed inhibitory innervation has not been established. In monogastric animals oesophageal and pharyngeal mechanical stimulation evokes relaxation of fundal smooth muscle (Cannon and Lieb, 1911; Lind, Duthie, Schlegel and Code, 1961; Abrahamson and Jansson, 1969). Martinson (1965b) suggests that the efferent limb of this reflex is provided by the vagal non-cholinergic non-adrenergic inhibitory fibres described by Jansson and Martinson (1965). It is suggested that this relaxation ('receptive relaxation') allows maintenance of stomach pressure within limits during ingestion and deglutition (Cannon and Lieb, 1911; Lind, Duthie , Schegel and Code, 1961). Such a mechanism is likely to be vestigial in adult ruminants as ingestion and deglutition does not result in immediate increase in the rate of digesta flow to the abomasum (Wyburn, 1980). If a feed-forward inhibitory mechanism exists whereby abomasal body relaxation is brought about prior to arrival of digesta in the abomasum it is reasonable to postulate that the sensory receptors involved are located in the reticulum as reticular contractions propel digesta to the omasum and

abomasum (Wyburn, 1980). It was decided to test for the possible existence of such a feed-forward inhibitory mechanism by excitation of mechanoreceptors in the reticulum. A control was required to allow differentiation between the effects of reticulum mechanoreceptor stimulation and the 'space' effects of reticular distension, which may include passive distortion of the abomasal body wall and activation of mechanoreceptors in, for example, the diaphragm, abomasum and parietal peritoneum. To this end an inflatable balloon was placed in the peritoneal cavity between the reticulum and the diaphragm.

PROTOCOL.

Intra-reticular and intra-abdominal balloons were inflated with 100-1500 ml of air in 100 ml increments in four sheep. Changes in abomasal body pressure produced by each of 35 inflations of the intra-reticular balloon and 25 inflations of the intra-abdominal balloon with volumes between 500 and 1500 ml were recorded and analysed. To enable group analysis of the experiments the percentage change induced in body pressure by inflation of either balloon was calculated.

Inflation of the intra-abdominal balloon simulated the mechanical effects of reticular distension with little stimulation of reticulum mechanoreceptors. Reticular distension by means of an intra-reticular balloon stimulated reticulum mechanoreceptors but did not transfer digesta from reticulum to abomasum. Differences in effect

on abomasal body tone produced by inflation of intra-reticular as opposed to intra-abdominal balloons were therefore ascribed to activation of reticulum mechanoreceptors.

RESULTS.

Inflation of either the intra-reticular balloon or the intra-abdominal balloon with volumes of less than 500 ml did not affect the motor profile of the abomasal body. In all experiments changes in body pressure and percentage body pressure produced by inflation of the intra-reticular balloon 500-1500 ml of air did not differ significantly from changes produced by inflation of the intra-abdominal balloon with 500-1500 ml of air. When inflation of either balloon produced a pressure increase in the body, the period of increased pressure coincided with the period of balloon inflation. Inflation of either balloon produced similar changes in the e.m.g. activity of the body (fig. 14).

The body pressure and e.m.g. response to each of 23 inflations of the intra-reticular balloon and 23 inflations of the intra-abdominal balloon were analysed. The integrated e.m.g. response to balloon inflation was classified as 'decreased', 'increased' or 'no change'. No consistent response to inflation of either the intra-reticular balloon or the intra-abdominal balloon was seen. Eight reticular balloon inflations (34.7%) produced increases in body pressure with no change in e.m.g. activity; seven inflations (30.5%) produced no change in

body pressure or e.m.g. activity; four inflations (17.4%) produced concurrent increases in body pressure and e.m.g. activity; three inflations (13.1%) produced a decrease in body e.m.g. activity with no change in body pressure; one inflation produced an increase in body pressure and a decrease in body e.m.g. activity. Of the intra-abdominal balloon inflations eleven (47.8%) produced an increase in body pressure with no change in the e.m.g. activity; three (13.1%) produced no change in abomasal body pressure or e.m.g. activity; four (17.3%) produced a decrease in body e.m.g. activity with no change in body pressure; three (13.1%) produced concurrent increases in body pressure and e.m.g. activity; two (8.7%) produced an increase in body e.m.g. activity and no change in body pressure.

No correlation was found between intra-reticular balloon volume and body pressure or percentage change in body pressure ($r = 0.017$ and $r = 0.075$ respectively). Similarily, there was no correlation between intra-abdominal balloon pressure and body pressure or percentage change in body pressure ($r = -0.09$ and $r = -0.05$ respectively). Regression analysis showed no significant slope when change in body pressure or percentage change in body pressure were plotted against intra-reticular volume, or when change in body pressure or percentage change in body pressure were plotted against intra-abdominal balloon volume (fig. 13).

DISCUSSION.

Previous reports of the association between reticulum and

abomasal motility (Czepa and Stigler, 1929; Magee, 1932; Kryzwanek and Quast, 1937; Phillipson, 1939; Ota, Ohga and Nakazoto, 1965) have come from records taken in conscious standing animals with on-going contractile activity of the reticulo-rumen, making investigation of the possible mechanisms of reticulo-abomasal interaction difficult. Possible mechanisms by which reticulum movement may affect abomasal body motility are:-

1. Activation of mechanoreceptors in the reticulum eliciting a reticulo-abomasal reflex.

2. The juxtaposition of the reticulum and abomasum is such that reticulum movement causes passive movement of the abomasum.

3. Reticular contraction moves digesta into the abomasum so evoking abomaso-abomasal reflexes.

4. A combination of these mechanisms.

Two populations of mechanoreceptor have been described in the reticulum; intra-mural 'in-series' tension receptors (Iggo 1955, 1956, Leek 1969), and epithelial receptors responsive to light brushing of the epithelium (Leek and Harding, 1975). The major sensory effect of inflation by an intra-reticular balloon is likely to be excitation of the 'in-series' tension receptors although contact between the balloon and localized areas of the reticulum wall may also excite the epithelial receptors. A more natural stimulus to the 'in-series' tension receptors would have been achieved by allowing the reticulum to contract onto the intra-

reticular balloon (Leek, 1969). This was not done as no adequate control could be devised, and abomasal manipulation has strong inhibitory effects on reticulum motility (Titchen, 1958).

The effects on abomasal body tone of intra-reticular and intra-abdominal balloon inflation were compared over a range of volumes. Although inflation of either balloon produced changes in abomasal body tone no consistent pattern of response was identified. No significant difference was found between the abomasal body responses produced by intra-reticular and intra-abdominal balloon inflation. These results, therefore, provide no evidence for the existence of a feed-forward reflex whereby abomasal body relaxation is brought about by activation of reticulum mechanoreceptors. The function of the vagal inhibitory innervation of the abomasal body (Chapter 4) therefore does not appear to be involved in feed-forward inhibition of the body by the reticulum. It is possible that a feed-forward inhibition of the abomasal body is instigated by activation of sensory receptors elsewhere, for example in the omasum. Stevens, Sellers and Spurrell (1960) report association between omasal canal and abomasal body motility in the ox. In monogastric animals stomach distension elicits stomach relaxation in a vagally-dependent manner (Abrahamsson, 1973a, 1973b; Andrews, Grundy and Lawes, 1980). No evidence was found for an equivalent reflex in the abomasum (Chapter 5).

The llama is a grazing animal with a compound stomach

consisting of four compartments, C1-C4, which can be compared to the rumen, reticulum, omasum and abomasum of the sheep respectively (Engelhardt, Ali and Wipper, 1979; Luciano, Reale and Engelhardt, 1980). Gregory, Heller and Engelhardt (1985) looked at the effect of distension of each of the four stomach compartments of the llama on the motility of the other stomach compartments. The motility of C4 (abomasum-equivalent) was not affected by distension of C1, C2 or C3. Distension of C4 inhibited the motility of C1, C2 and C3. This is a parallel situation to that in the sheep: no evidence was found in these experiments for a feedforward reflex from the reticulum to the abomasum, and abomasal distension inhibits reticuloruminal contractile activity (Titchen, 1958). Thus it is possible that the reflex mechanisms controlling the stomach-equivalent of ruminants evolved along quite different lines than the reflex mechanisms controlling stomach pressure in monogastric animals: monogastric animals depend on feedforward reflexes allowing the stomach to relax to accommodate incoming ingesta; ruminants depend on feedback reflexes to limit the amount of incoming digesta.

Reticular distension between 500 and 1500 ml increased abomasal body pressure in 23 out of 35 trials (65.7%). As there was no transfer of digesta from the reticulum to the abomasum in these experiments, nor any evidence of a reticulo-abomasal body reflex it is likely that the increase in body pressure was due to impingement of the reticulum on the abomasal body wall. Phillipson (1939) reported that the reticulorumen motility was reflected in

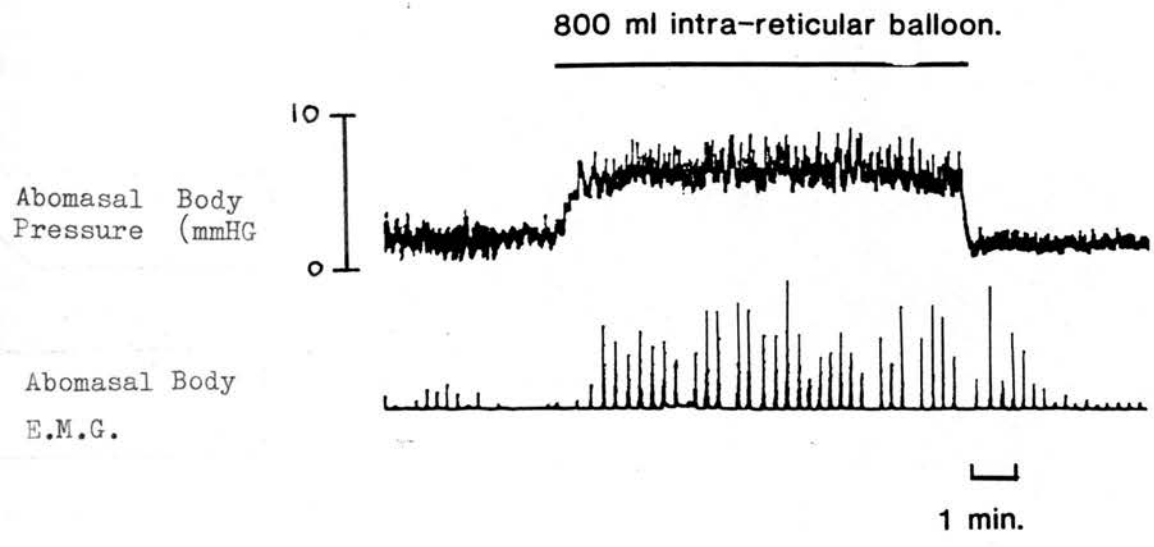
pressure recordings taken from the abomasal body. Such interaction between the abomasal body and the surrounding viscera may be functionally important in the transfer of digesta from the body to the antrum. Reticulo-rumen motility may thus be involved in transfer of digesta from the abomasal body to the antrum (Phillipson, 1939).

Figure 14.

No significant differences were found in the effects on abomasal body pressure and e.m.g. of inflation of intra-abdominal (n = 25) and intra-reticular (n = 35) balloons.

'A' shows the effect on abomasal body pressure (upper trace) and integrated e.m.g. (lower trace) of inflation with air of an intra-reticular balloon to 800 ml. The bar corresponds to the period of reticular inflation. 'B' shows the effect on abomasal body pressure (upper trace) and integrated e.m.g. (lower trace) of inflation with air of an intra-abdominal balloon to 800 ml. The bar corresponds to the period of intra-abdominal balloon inflation. In both 'A' and 'B' balloon inflation caused an increase in body e.m.g. which persisted for 1-6 min after balloon deflation. This was not a consistent finding.

A


800 ml intra-reticular balloon.
10
0
Abomasal Body Pressure (mmHG
Abomasal Body E.M.G.
1 min.

B

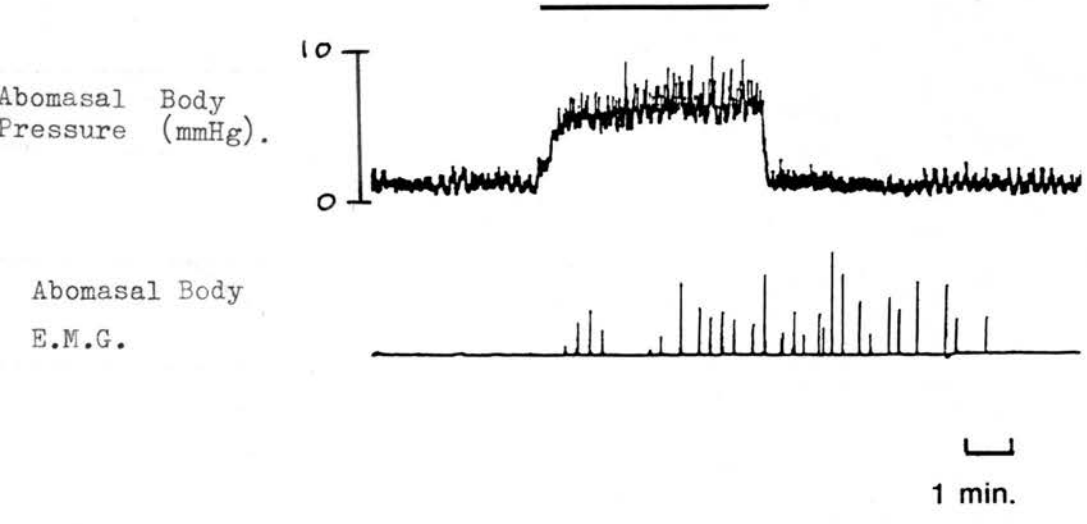
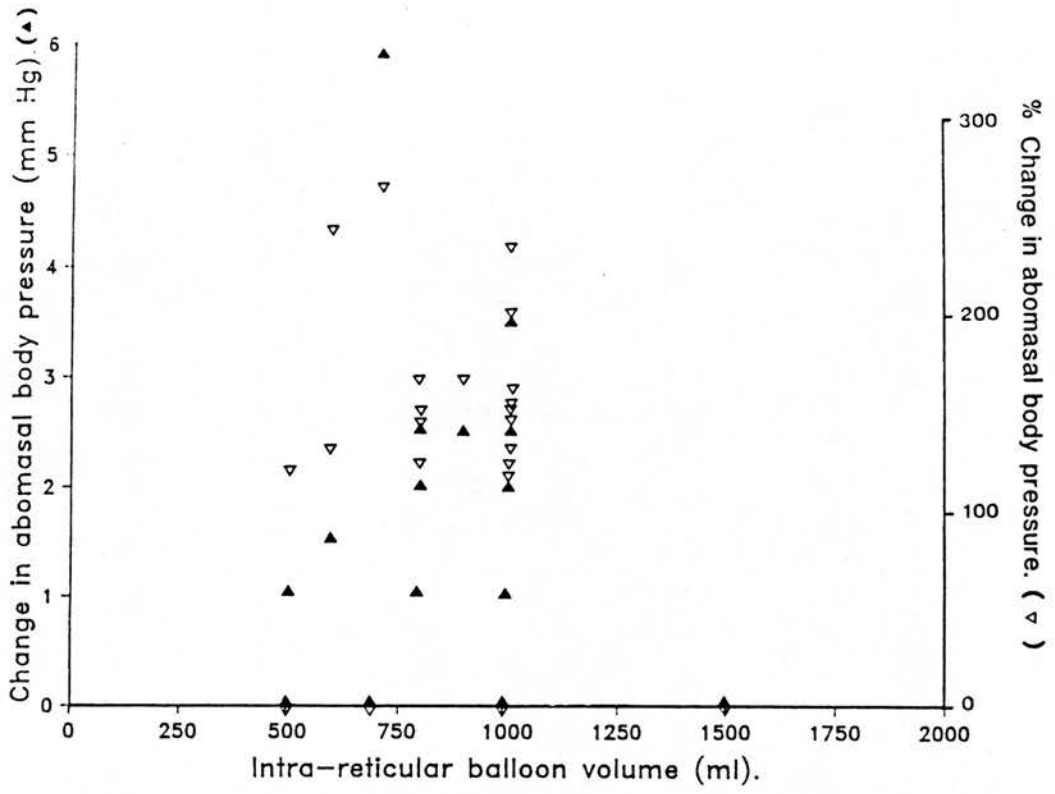

800ml intra-abdominal balloon.
10
0
Abomasal Body Pressure (mmHg).
Abomasal Body E.M.G.
1 min.

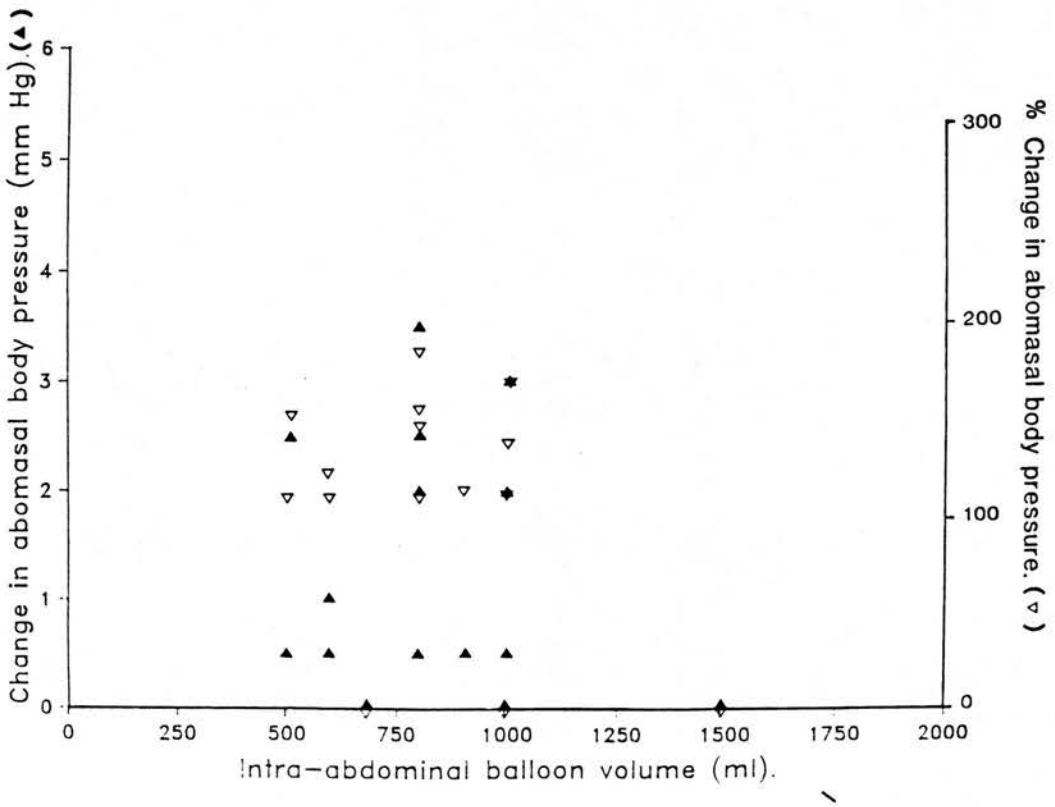
Figure 15.

Graphs of change in abomasal body pressure (▲) and percentage change (Δ) in abomasal body pressure against (A) intra-reticular balloon volume and (B) intra-abdominal balloon pressure. No significant correlation was found in either case.

A


Change in abomasal body pressure (mm Hg).(▲)
% Change in abomasal body pressure. (▽)
Intra-reticular balloon volume (ml).
0
250
500
750
1000
1250
1500
1750
2000
1
2
3
4
5
6
100
200
300

B


Change in abomasal body pressure (mm Hg).(▲)
% Change in abomasal body pressure. (▽)
Intra-abdominal balloon volume (ml).
0
250
500
750
1000
1250
1500
1750
2000
1
2
3
4
5
6
100
200
300

CHAPTER SEVEN.

DISCHARGE CHARACTERISTICS OF SINGLE EFFERENT UNITS INNERVATING ABOMASAL ANTRAL STRUCTURES.

INTRODUCTION.

The discharge characteristics of single efferents units associated with both the reticulorumen (Iggo and Leek 1967a, 1967b; Harding and Leek 1971, 1972b, 1973) and the monogastric stomach (Davison and Grundy, 1976, 1977, 1978a, 1978b; Andrews, Duthie, Fussey and Mellersh, 1978; Andrews, Salih and Scratcherd, 1978; Grundy, Salih and Scratcherd, 1981; Blackshaw, Grundy and Scratcherd, 1987) have been extensively reported. Discharge characteristics of efferents identified as associated with the ruminant abomasum have not previously been described.

Previous reports of efferent activity associated with the ruminant forestomach or monogastric stomach have involved sampling unitary activity at either the cervical or central levels. Such techniques are unable to locate efferent function or destination. For this reason it was decided to sample efferent unit activity at the level of the abomasal antrum. Antral efferent units were chosen in preference to those of the body for 3 reasons:-

1). To try to find a neural response that correlated with the abomasal body-antral excitatory reflex described in Chapter 5 of this work.

2). Antral motility is both more pronounced and more

regular than body motility, so antral motility rhythms in unitary discharge may be more easily discernable than body motility rhythms. The variability of antral motility patterns seen in ostensibly identical abomasal preparations suggests a degree of extrinsic regulation of antral motility.

3). As antral and body efferents share common nerve trunks, a sample of exclusively body fibres could not be obtained.

Recent reports have indicated extensive central convergence of central regulating systems of diverse function and afferent fibres from disparate sources onto motoneurones (Kollai and Koizumi, 1979, 1980; Grundy, Salih and Scratcherd, 1981; Gebber, 1984). This calls into question the practice of:

a). in recordings made at the cervical level of excluding fibres with cardiovascular- or ventilation-related rhythms from samples of fibres studied for gastro-intestinal association.

b). techniques involving efferent classification according to the association of their discharge with a system-related rhythm (eg. the cardiac cycle) or a specific event (eg. the contraction of a viscus).

Thus the opportunity was taken to look for units with the characteristics classically ascribed to cardiovascular efferent units plus the characteristics of efferent units associated with the gastro-intestinal tract.

RESULTS

The activity of 33 units from 12 sheep was recorded.

General properties of the units.

Conduction velocities of fibres ranged from 0.91-2.33 m s^{-1}. (Mean +/- s.d. = 1.49 +/- 0.95 m s^{-1}, n = 21). The spontaneous discharge rate ranged from 0.3-25.6 impulses s^{-1} (mean +/- s.d. = 4.0 +/- 5.4 impulses s^{-1}). All units had an irregular spontaneous discharge pattern. No overt cardiovascular, respiratory or antral motility rhythm was discernable in the discharge of any single unit without post stimulus time histogram (PSTH) analysis. The integrated recording of a multifibre strand discharge with obvious arterial pressure association is shown. (fig. 16).

Effect of adrenaline injection.

a). Arterial Pressure.

The resting systolic arterial pressure of the preparations, measured from a catheter in the femoral artery (with its tip in the distal aorta), ranged from 60-135 mmHg. Intra-venous injection of 100 ug adrenaline over 30-60 s caused increases of 50-160 mmHg in systolic arterial pressure (mean increase +/- s.d was 96.3 +/- 32.6 mmHg). Percentage increase range was 143.0-325.0% %, (mean percentage increase +/- s.d. was 225.4 +/- 56.0 %). Mean rate of increase +/- s.d. of the adrenaline-induced rise in systolic arterial pressure was 2.6 +/- 1.0 mmHg s^{-1}. Blood pressure returned to resting levels within 4 min of the

start of arterial pressure increase (fig. 17, 18).

b). Abomasal Motility.

Intra-venous adrenaline injection inhibited body and antrum contractile activity and caused a fall in antral tone (fig. 17). The duration of inhibition of abomasal motility could be longer than, shorter than, or equal to the duration of the cardiopressor effect of adrenaline injection. Changes in unitary discharge induced by adrenaline injection followed the time course of arterial pressure changes rather than that of abomasal motility changes.

c). Unitary discharge.

Injection of 100 ug of adrenaline into the femoral vein affected the discharge of units in one of three ways; abolition of discharge, reduction in discharge rate, or increase in discharge rate.

1. Intra-venous adrenaline injection abolished the discharge of 14 (42.4%) units (fig. 18 A). The activity of these units stopped at the onset of arterial pressure increase (fig. 18 A). The discharge activity returned gradually as the arterial pressure returned towards pre-injection levels.

2. Intra-venous adrenaline injection reduced the discharge rate of 6 (18.2 %) units (fig. 18 B). Percentage fall in discharge rate correlated with percentage increase in arterial pressure ($r = 0.77$) (fig. 19) but was unrelated to the rate of arterial pressure increase. The drop in

discharge rate in these fibres occurred abruptly as the arterial pressure began to increase (fig. 18 B). Discharge activity returned towards pre-stimulus levels as the arterial pressure returned to pre-injection values.

3. Intra-venous adrenaline injection increased the discharge rate of 7 (21.2%) units (fig. 18 C). No correlation could be identified between percentage increase in discharge rate and either the percentage increase in arterial pressure, or the rate of change of arterial pressure. ($r = 0.3$ and $r = -0.1$ respectively). Percentage increase in the discharge rate ranged from 155%-924% of the resting rate. The increase in discharge rate peaked at the same time as the arterial pressure peak (fig. 18 C) and returned with the arterial pressure to pre-stimulus levels.

The discharge of 6 units (18.2%) was not modulated by intra-venous adrenaline injection (fig. 18 D).

No significant difference was found in the conduction velocities or spontaneous discharge rates of these groups. As the groups had been identified on the basis of their response to a stimulus of increase in arterial pressure, the parameters (mean induced arterial pressure increase, percentage arterial pressure increase and rate of arterial pressure increase) of the stimuli for each group were compared. There was no evidence of a significant difference between the stimuli applied to each group.

Relationship of resting arterial pressure to spontaneous discharge rate.

The spontaneous discharge rates of all units were plotted against the resting arterial pressure recorded at the time of each unit recording. No correlation was found. Also, the spontaneous discharge rates of the units in each of the groups classified by their response to arterial baroreceptor stimulation were plotted against the resting systolic arterial pressure. No correlation was found between spontaneous discharge and resting arterial pressure of any of the groups.

Response of efferents to stimulation of antral mechanoreceptors.

Modulation of the activity of 27 efferent units was attempted by inflation of an antral balloon with 50 ml of air in 5-10 ml increments. No modulation of efferent discharge was achieved by this method.

Response of efferents to stimulation of abomasal body mechanoreceptors.

The activity of 10 (32.25 %) out of 31 units was abolished or decreased by inflation with air of a balloon in the abomasal body. The mean conduction velocity (1.49 m s^{-1}, s.d. = 0.41 m s^{-1}) and spontaneous discharge rate (3.1 impulses s^{-1}, s.d. = 2.87 impulses s^{-1}) of the body inflation sensitive units did not differ significantly from that of the body inflation-insensitive units. No unit was found whose activity was increased by body inflation.

Six of the 10 units modulated by inflation of the body balloon responded by complete cessation of discharge activity (fig. 20). The minimum volume of inflation required for this 'switching off' of discharge activity ranged from 10 to 250 ml. The discharge activity of three of these units returned abruptly on deflation of the body balloon; the discharge of the remaining three units returned gradually over 30-200 s.

Four of the 10 units sensitive to inflation of the body balloon showed a stepped reciprocal decrease in discharge rate with stepped inflation of the abomasal body to 100 ml (fig. 21 A). By pooling the results of these units, and taking the spontaneous discharge rate for each unit as 100 %, the percentage decrease in discharge rate was plotted against body volume, body pressure and percentage body pressure. Good correlation was found between the percentage discharge rate and both body volume and percentage body pressure ($r = -0.76$ and $r = -0.79$ respectively); however body pressure showed the highest correlation with percentage discharge rate ($r = -0.85$, fig. 21 B). The activity of 3 of these units gradually returned to a steady level over 30-200 s after deflation of the body balloon; the discharge activity of the remaining unit returned abruptly to a steady level on deflation of the body balloon.

All the units that responded to inflation of the body balloon also responded to the increase in arterial pressure coincident with intra-venous injection of adrenaline. The

discharge of 7 units stopped with arterial pressure increase; the discharge of 1 unit was reduced by arterial pressure increase; the discharge of 2 units increased as arterial pressure increased. No unit sensitive to body inflation alone was found.

Post stimulus time histogram analysis.

a). Antral rhythmicity.

The possibility of a temporal relationship between the discharge of 25 efferent units in the dorsal and ventral continuations of the abdominal vagi close to the antrum and the electrical activity of the antrum was investigated. Post stimulus time histograms of discharge activity with respect to the beginning of the electrical burst activity of the antral e.m.g. were constructed. An estimation of the 'degree of antral rhythmicity' was obtained by taking the difference in the maximum and minimum activity (each determined over a 6-bin period) of the e.m.g. interval histogram as a percentage of the mean activity of the e.m.g interval histogram in the same manner as the degree of cardiac rhythmicity was calculated from the post R-wave histogram (see below). The 'degree of antral rhythmicity' ranged from 27.0 to 220.4 %; the mean antral rhythmicity (+/- s.d.) of all units was 73.2 (+/- 41.5 %). The mean 'degree of antral rhythmicity' of the units whose activity was modulated by abomasal inflation (73.6 %, s.d. = 55.9 %) did not differ significantly from that of the units whose activity was not modulated by abomasal inflation (mean +/- s.d. = 73.1 +/- 29.8 %).

Observation of the shape of the e.m.g. interval histograms indicated the temporal relationship of the discharge of any unit ('antral periodicity') with the antral e.m.g. cycle. Two patterns of antral periodicity were seen: 7 units had three periods of peak discharge per antral cycle (3:1 antral periodicity); 11 units had two periods of peak discharge per antral cycle (2:1 antral periodicity) (fig. 24). No pattern was obvious in the e.m.g. histogram of 7 units.

b). Cardiac rhythmicity.

The cardiac rhythmicity of the activity of 25 of the units isolated from the abdominal continuations of the dorsal and ventral vagi was determined with respect to the R-wave of the e.c.g. from post R-wave histograms. The 'degree of cardiac rhythmicity' for each unit was calculated by taking the difference in the minimum and maximum activity (each calculated over a 6-bin period) of a post R-wave histogram as a percentage of the mean activity of the R-wave histogram (fig. 22) (Hainsworth and Linden, 1979). The 'degree of cardiac rhythmicity' of the 25 units ranged from 13.4-208.9 %; the mean +/- s.d. was 76.2 +/- 51.5 %. No significant difference was found between the mean 'degree of cardiac rhythmicity' of the four groups of units classified by their response to stimulation of arterial baroreceptors.

Observation of the shape of the post R-wave histograms of these units gave an indication of the temporal relationship of the discharge ('cardiac periodicity') of any unit with

the cardiac cycle. Three patterns of cardiac periodicity were seen: the activity of 15 units peaked at one stage per cardiac cycle (1:1 cardiac periodicity); the activity of 7 units peaked at two stages per cardiac cycle (2:1 cardiac periodicity); the activity of 2 units peaked at three stages per cardiac cycle (3:1 cardiac periodicity) (fig. 23). No periodicity was obvious in the post R-wave histogram of 1 unit. The peak discharge of the units came at different stages of the cardiac cycle. Of the 15 units with a 1:1 periodicity with the cardiac cycle the peak activity of 6 units occurred just before the R-wave; the peak activity of 3 units occurred at the R-wave; the peak activity of 6 units occurred in the middle two-thirds of the R-wave histogram. Of the 7 units with a 2:1 periodicity with the cardiac both peaks occurred in the intra-R-wave interval of 4 units; in 3 units one peak co-incided with the R-wave. The type of periodicity with respect to the cardiac cycle exhibited by any unit was not related to the response of the unit to adrenaline injection.

DISCUSSION.

The prime objective of the recording technique employed in these experiments was to sample the activity of efferent neurones innervating the tissues of the abomasal antrum. This was achieved by recording the activity of nerves sectioned within 2 cm of the antrum. This technique imposed limitations. Due to the peculiarities of the extrinsic innervation of the sheep antrum, where splanchnic and vagal fibres co-exist in the so-called abdominal continuations of

the dorsal and ventral vagi (Habel, 1956), vagal and splanchnic fibres could not be differentiated. It is likely that a mixed population of pre- and post-ganglionic fibres was sampled because this nerve contains sympathetic postganglionic and vagal preganglionic fibres (Habel, 1956). Sympathetic cholinergic pre-ganglionic fibres have been identified at this level in other species (Semba and Hiroaka, 1957) so their presence in the sheep cannot be discounted. As Habel (1956) described vagal fibres to the coeliac ganglion in the sheep the possibility of vagal postganglionic fibres, with their cell bodies in the coeliac ganglia, occurring at this level can neither be discounted. In any nerve recording technique involving dissection of the nerve there is a possibility of ephaptic recordings. The juxtaposition of the recording site and the abomasal body restricted the volume of body inflation that could be used as a stimulus to modulate efferent activity. Although the location of the recording site was such as to ensure that the fibres studied were innervating antral structures, closer identification of precise efferent destination (vascular, secretion-associated, or motility-associated smooth muscle) was not possible. These limitations not withstanding, valid and valuable conclusions can be drawn from the results obtained.

The range of conduction velocities identified the nerves as C-fibres. This is typical of sympathetic postganglionic fibres and vagal preganglionic fibres at this abdominal level. The discharge rates of the fibres were within the C-

fibre range (Iggo, 1958).

The activity of 10 antral efferent units was decreased or abolished by inflation of the abomasal body. This represented 32.5 % of units tested. Because of the restrictions on the volume to which the body could be inflated without disturbing the recording electrodes, this figure is probably an under-representation of the proportion of units whose activity may be modulated by body inflation.

The conduction velocity of the body inflation-modulated units identified them as C-fibres and did not differ significantly from the conduction velocity of the fibres whose activity was not modulated by body inflation. This contrasts with the reports of Bahr, Bartel, Blumberg and Janig (1986a, 1986c) who found that the conduction velocity of sympathetic neurones whose activity could be modulated by mechanical manipulation of the pelvic viscera of the cat was on average 4 times greater than the conduction velocity of fibres whose activity was not modulated by mechanical manipulation of the pelvic viscera. However, they were dealing with preganglionic units from the lumbar sympathetic trunk, which has a higher proportion of myelinated fibres than the abdominal continuations of the dorsal and ventral vagi of the sheep.

There is evidence for a sensory apparatus in the abomasum capable of modulating efferent activity. Inflation-sensitive receptors in the abomasal wall are implicated in studies of abomasal reflexes by Phillipson (1939) and

Titchen (1958). Abomasal pyloric distension also modulates the activity of interneurones and vagal preganglionic motorneurones (with reticuloruminal function) in the dorsal vagal nucleus of the sheep (Harding and Leek, 1972b).

The amplitude and frequency of antral contractions determine the rate of abomasal emptying. Evidence has been presented to suggest that the abomasal body exerts an inhibitory influence on the amplitude of antral contractions by intrinsic pathways (Chapter 3). A vago-vagal body-antral reflex where inflation of the body increases the amplitude of antral contraction has also been described (Chapter 5). The present experiments have identified antral efferent units whose activity is decreased by body inflation. If these units are instrumental in bringing about the extrinsically-mediated reflex increase in antral contraction amplitude it is reasonable to assume that they are inhibitory in nature. Thus a reflex increase in antral contraction amplitude may result from a decreased in inhibitory efferent drive rather than an increase in excitatory efferent drive to the antrum. The results of electrical stimulation of the cut peripheral end of the cervical vagus (Chapter 4) indicate the existence of a population of vagal inhibitory fibres that influences the amplitude of contraction of the antrum in the sheep.

Abomasal body distension did not increase the discharge rate of any of the units tested.

No modulation of antral efferent unitary activity was

achieved by inflation of the antrum, nor did increase in antral pressure elicit an antro-antral excitatory reflex in the sheep (Chapter 5). An antro-antral excitatory reflex has been described in the ferret (Grundy, Hutson and Scratcherd, 1986) where inflation of an antral balloon increases the amplitude of antral contraction. However, the reflex is mediated through enteric neurones; the vagus plays only a permissive role.

These results differ from reports of units from the cervical vagus of monogastric animals whose activity may be modulated by inflation of the monogastric stomach. Davison and Grundy (1976, 1977, 1978a, 1978b) describe four types of unit response, recorded at the cervical level, to stomach inflation in the rat. Type I unit discharge increases with stomach inflation; type II unit discharge decreases with stomach inflation; type III unit discharge increases with moderate stomach inflation, but decreases at higher inflation volumes; type IV unit discharge decreases with moderate stomach distension but increases with higher volumes of inflation. Unit responses, also recorded at the cervical level, corresponding to types I, II and III have been identified in the ferret (Grundy, Salih and Scratcherd, 1981). In the experiments described here in sheep only antral efferent units corresponding to monogastric type II were identified (although the moderate levels of inflation feasible in these experiments may not have been sufficient to differentiate between type II and type IV units). However there is no good evidence (nor do the authors claim) that the units described in the rat and

ferret experiments are stomach (much less antral) efferents. Also, the experiments in the rat and ferret involved whole stomach inflation; body and antral effects were not isolated. Although only moderate levels of body distension were possible in the sheep experiments it is likely that if antral efferent units with responses equivalent to monogastric type I and III were present in the sheep they would have been identified.

Davison and Grundy (1978a) described a sub-population of type I units (type Ic) with no tonic activity. These units fired only during contractions of the stomach. No equivalent units were identified in the sheep.

Intra-venous injection of adrenaline brings about, inter alia, an increase in systemic arterial pressure by a combination of increasing cardiac contractility and by acting directly on vascular smooth muscle receptors to cause an increase in peripheral resistance. The resulting increase in arterial pressure is detected by the arterial baroreceptors. In this experiment three populations of efferent unit were identified by their response when blood pressure rose due to adrenaline injection : a non-responsive group, a group whose discharge rate was decreased or abolished, and a group whose discharge rate was increased.

The behaviour of the population of fibres whose discharge rate was reduced or abolished by intra-venous adrenaline injection was typical of sympathetic visceral vasoconstrictor ('VVC') neurones (Bahr, Bartel, Blumberg and Janig 1986a, 1986b, 1986c; Bartel, Blumberg and

Janig, 1986). The fibres identified in the present study had similar spontaneous discharge rates and conduction velocity ranges to described VVC neurones. Increase in arterial pressure increases the tonic inhibitory drive of aortic (vagal) baroreceptor afferents and carotid (glossopharyngeal) baroreptor afferents to the medullary units regulating sympathetic vasoconstrictor activity. This increase in inhibitory drive reduces or abolishes the discharge of sympathetic vasoconstrictor units. In this study units whose discharge was reduced, and units whose discharge was abolished by arterial baroreceptor stimulation, are likely to be sympathetic VVC neurones; they were grouped separately only for purposes of analysis. It was characteristic of these units, and the units described by Bahr et al (1986a, 1986b, 1986c) and Bartel et al (1986) that the discharge rate decreased abruptly to a lower level at the start of arterial pressure increase and stabilized at this new low level before the full magnitude of the arterial pressure rise was apparent. Thus it is difficult to understand the mechanism which establishes the correlation ($r = 0.77$) between the percentage change in arterial pressure and the percentage change in discharge rate, especially as no correlation was found between the rate of change in arterial pressure and the change or percentage change in discharge activity.

The activity of 7 units was increased by injection of adrenaline. The function of these units is unknown. They may have cardiovascular function; the change in discharge rate closely mirrored change in arterial pressure. No

reference has been found in the literature to either a population of visceral efferent fibres whose activity is increased by sti increase in blood pressure, or to a population of visceral vasodilator fibres. If these fibres do have vasodilator function they differ from the sympathetic vasodilator fibres innervating arterioles of skeletal muscle; the somatic vasodilator fibres are controlled by cortico-hypothalamic-reticulo-spinal pathways and do not respond to stimulation of arterial baroreceptors (Wright, 1982). A suggested function of the somatic vasodilator fibres is the provision of a 'safety valve' mechanism by opening arterial-venous shunts in skeletal muscle (Wright, 1982). It is feasible that the population of visceral fibres that responded to adrenaline injection by increasing their discharge rate provide a similar vasodilatory 'safety valve' mechanism for the splanchnic circulation. The time course of the increase in discharge rate of the units stimulated by adrenaline injection followed the time course of the increase in arterial pressure. This contrasted markedly with the response characteristics of the 'VVC' units whose discharge rate was decreased by increase in arterial pressure. Thus although the activity of both types of unit may be modulated by afferent input from the arterial baroreceptors, the units are subject to different mechanisms of central control. Somatosympathetic vasoconstrictor and vasodilator fibres are also subject to different central control mechanisms (Wright, 1982).

It is possible that the increase in discharge rate of

units caused by adrenaline injection is secondary to a non-cardiovascular effect of the adrenaline such as the effect on antral motility. Intra-arterial injection over 30 s of 10 ug of adrenaline close to the antrum causes an increase in the discharge rate of antral tension receptors (Cottrell, D.F. and Reynolds, G.W., personal communication). This could cause a reflex increase in the discharge rate of antral efferent units. The activity of only one of the units whose activity was increased by adrenaline injection was modulated by inflation of the abomasal body.

Unitary discharge was examined for temporal relationship with the cardiac cycle. No overt cardiac rhythm was apparent without P.S.T.H. analysis in any single unit. Multiunit recordings did have a discernable cardiac rhythm. This has been reported in most descriptions of both somatic and visceral sympathetic multifibre recordings (Adrian, Bronk and Phillips, 1932; Gernandt, Liljestrand and Zotterman, 1946; Cohen and Gootman, 1970; Koizumi, Seller, Kauffman and Mc Chandler Brooks, 1971; Bower, 1975; Nosaka, Sato and Shimada, 1980). One reason for difficulty in discerning overt cardiac rhythms in single unit activity patterns is that the low spontaneous discharge rate is often less than the heart rate.

Post stimulus time histogram analysis with respect to the R-wave showed that most units had some degree of 'cardiac rhythmicity.' The mean degree of cardiac rhythmicity (76.2 %, s.d. = 51.5 %) of these units is less than that

described by Bahr et al for the lumbar sympathetic preganglionic units of the cat. The cardiac rhythmicity in sympathetic neurone discharge is thought to arise as a result of the combined influence of baroreceptor afferent tonic input, waxing and waning in phase with systole and diastole, and the inherent discharge rhythm of independent oscillation circuits in the brainstem (Barman and Gebber, 1976). Three patterns of cardiac periodicity were observed in the sheep: a 3:1, a 2:1 and a 1:1 discharge peak:cardiac cycle ratio. Cardiac periodicities in visceral sympathetic recordings of up to 5:1 have been described (Barman, 1984). No descriptions of PSTH analysis with respect to the cardiac cycle of vagal units either to the abomasum or monogastric stomach, or whose activity is modulated by mechanical stimulation of the abomasum or monogastric stomach have been found in the literature.

Post stimulus time histogram analysis, with respect to the start of electrical burst activity of the antral e.m.g., showed that the units exhibited a degree of 'antral rhythmicity' very similar in range and mean to the degree of cardiac rhythmicity. Two patterns of antral periodicity (3:1 and 2:1) were observed in the e.m.g.-related post stimulus time histograms. No reference to a P.S.T.H. analysis of efferent activity with respect to any gastro-intestinal rhythm has been found for any species. It is not possible to say from this study if the antral rhythmicity displayed by antral efferent unit discharge is central or peripheral in origin. It is possible that the mechanism is analagous to the mechanism proposed by Barman (1984)

whereby sympathetic neurone discharge displays both ventilation and cardiac rhythmicities. He presents evidence for independent neuronal oscillation circuits, located in the brainstem and spinal cord, which determine the inherent rhythms of sympathetic neurone discharge. One of the oscillation circuits has a discharge rhythm related to cardiac rhythm; another has a slow cycle associated with ventilation. Because of coupling between the oscillation circuits many sympathetic neurones have discharge patterns with the rhythms of both oscillation circuits. Sympathetic neurone discharge is further modified by tonic afferent input from arterial baroreceptors and pulmonary stretch receptors. Also, Kollai and Koizumi (1979, 1980) present evidence that the presence of both cardiac and respiratory rhythms in the discharge of cardiac parasympathetic fibres is due to complex central interaction of independent oscillation circuits with either cardiac or ventilation rhythms, and tonic afferent drive to the nucleus of the solitary tract and the nucleus ambiguus. Harding and Leek (1971) working on the hindbrain of the sheep have described a system of 'interneurones' with an oscillating discharge periodicity of approximately 1 min concerned with the regulation of reticulo-ruminal motility. Grundy, Salih and Scratcherd (1981) propose the existence of a central 'reverberating circuit' maintaining discharge in putative gastrointestinal efferents in the ferret. It is possible that a similar oscillation circuit with a discharge pattern related to that of antral contractile activity, together with the modifying tonic input from antral afferent discharge, in phase with antral contractile activity

(Cottrell D.F. and Reynolds G.W., personal communication) impose an antral rhythmicity upon the discharge of antral efferents. There is evidence to suggest that the frequency of the basic electrical rhythm of gastrointestinal smooth muscle may be influenced by the enteric nervous system (Saunders and Smith, 1987). Recent evidence suggests that the frequency of the basic electrical rhythm may also be subject to central influence. Deloof and Rousseau (1985) and Deloof, Bennis and Rousseau (1987) have shown that the basic electrical rhythm of the antrum of the rabbit may be influenced by both vagal and splanchnic inputs. It is interesting to remember that muscle cells of the diaphragm adapt antral discharge rhythms when reinnervated by cross-suturing the cut central end of the left thoracic vagus to the cut peripheral end of the phrenic nerve (Moilin and Roman, 1978). Thus a 'chicken and egg' situation is presented; is it the inherent oscillation rhythm of the membrane of antral smooth muscle mirrored in antral afferent discharge that reflexly causes antral rhythmicity in antral efferent units, or does a centrally-arising rhythmicity in antral efferent unit discharge modify the rhythms of antral smooth muscle electrical activity? Or does the interaction of antral afferents and units from a 'central oscillation circuit' impose an antral rhythmicity without functional significance onto antral efferent discharge? Electrophysiological identification of appropriate oscillation circuits, recording of the discharge patterns of the appropriate central visceromotor sites before and after deafferentation (to unmask an

inherent rhythm, if present), and use of electrophysiological techniques to trace efferents from the hindbrain to the stomach (Andrews, Duthie, Fussey and Mellersh, 1978) are necessary to resolve this question.

A major difference between the results of these experiments and the results of experiments described in the literature is the degree of system-related discharge pattern overlap in the abomasal efferent units. The discharge of all units found in these experiments whose activity was modulated by inflation of the abomasal body was also modulated by injection of adrenaline.

No significant difference was found in the degree of modulation achieved by injection of adrenaline of units that were and units that were not modulated by abomasal body inflation. The units that displayed antral rhythmicities in P.S.T.H. analysis with respect to the start of the electrical burst activity of the antral e.m.g. also displayed cardiac rhythmicities in P.S.T.H. analysis with respect to the R-wave of the e.c.g. No significant difference was found in the 'degree of antral rhythmicity' displayed by units that were, and were not modulated by body inflation. Thus it was not possible to deduce efferent unit destination or function on the basis of either the system-related rhythms displayed in unitary discharge or on the basis of reflex response to selective stimulation of specific sensory pathways. This contrasts markedly with the results of studies on the discharge characteristics of lumbar sympathetic preganglionic (Bahr et al 1986a, 1986b, 1986c, 1986d) and

postganglionic (Janig, Schmidt, Schnitschler and Wesselman, 1986) fibres in the cat where two nearly discrete populations of fibres were found. One group (visceral vasoconstrictor or 'VVC' neurones) is responsive to stimulation of the arterial baroreceptors and displays temporal association of discharge with the cardiac cycle on P.S.T.H. analysis. The second group (motility regulating or 'MR' neurones) is responsive to mechanical stimulation of the pelvic viscera and perianal skin but does not display temporal association of discharge with the cardiac cycle. Only 14% of MR neurones also display VVC characteristics in the cat. This classification into 'VVC' and 'MR' is justifiable only where:

a) neurone populations as discrete as those described in the cat exist.

b) where the recording site is such as to allow identification of the efferent target organ.

The failure to identify discrete neuronal populations in the sample of antral efferent units in the sheep means that use of the VVC/MR classification awaits further investigation. Most reports on visceral efferent units whose activity is modulated by mechanical manipulation of the monogastric stomach have acknowledged the difficulty in determining efferent function by classifying the units according to reflex response rather than by ascribing function or destination (Davison and Grundy 1976, 1977, 1978a, 1978b; Grundy, Salih and Scratcherd, 1981; Blackshaw, Grundy and Scratcherd, 1987). This is not true for the studies of efferent activity related to reticulo-ruminal function.

Iggo and Leek (1967a, 1967b) and Harding and Leek (1971, 1972b) confidently ascribe reticulo-ruminal function to vagal motoneurones on the basis of the very obvious association of motoneurone discharge with both ongoing reticulo-ruminal cyclical activity and reflex modulation of reticulo-ruminal motility. The presence of such specific association coupled with the absence of overt discharge rhythms related to any other system, in particular the tonic activity characteristic of pulmonary and cardiac units, partly justifies their conclusions. In view of possible neural connections between the reticulorumen and other parts of the gastrointestinal tract the strength of their conclusions would have been enhanced by PSTH analysis of the efferent discharge for rhythms (or more specifically, the absence of rhythms) related to other systems, especially those of the abdominal viscera. It appears thus that in some instances the identification by PSTH analysis of system-related rhythms in the discharge pattern of an efferent unit is less useful and less meaningful than the demonstration of the absence of such rhythms from the discharge pattern of an efferent unit. This is especially true in the light of evidence in the ferret for central convergence of afferents from disparate regions of the gastrointestinal tract onto efferent units (Grundy, Salih and Scratcherd, 1981).

In sampling the cervical vagus for efferent units whose activity is modulated by mechanical stimulation of the gastro-intestinal tract it has been usual for units with cardiac or ventilation rhythms to be excluded from the

sample (Iggo and Leek, 1967a, 1967b; Davison and Grundy, 1978; Grundy, Salih and Scratcherd, 1981; Blackshaw, Grundy and Scratcherd, 1987). The degree of overlap of system-related discharge rhythms identified in the present study, and the recognized convergence on efferent systems of afferent fibres from disparate peripheral sources and central regulating circuits of diverse function, suggests that discarding units on the basis of system-related rhythms may result in some degree of bias of the efferent sample.

In these experiments the motor profile of the antrum was considered to be composed of a prevailing tone upon which contractions could be superimposed (Chapter 3). The tonic component of the antral motor profile is not very apparent as the antrum shows continuous contractile activity. Nevertheless it is tenable to consider the antral motor profile as consisting of tonic and contractile components as antral contraction amplitude could be altered without altering antral tone.

a). Transection of the abomasum into separate body and antral pouches increased antral contraction amplitude without affecting antral tone (Chapter 3).

b). Electrical stimulation of the peripheral end of the cut cervical vagus can either increase or decrease antral contraction amplitude without affecting antral tone (Chapter 4)

c). Abomasal body inflation increased the amplitude of antral contraction amplitude independently of its effect on

antral tone (Chapter 5).

Although no reflex modulation of antral tone was achieved, the presence of a tonic component to the antral motor profile was demonstrated by the marked fall in antral pouch tone produced by intravenous adrenaline injection (fig. 17). Thus antral tone may be maintained by activity of the intrinsic nervous system, and may be independent of extrinsic neural influences.

Figure 16.

A histogram of antral multi-unit efferent discharge activity s^{-1} (upper trace) on the same time axis as blood pressure (lower trace). Minimum discharge occurred during the rising phase of the Meyer waves of blood pressure; maximum discharge occurred during the falling phase of the Meyer waves of blood pressure. The recording of nerve activity was taken from a multifibre strand dissected from the abdominal continuation of the dorsal vagus within 2 cm of the abomasal antrum.

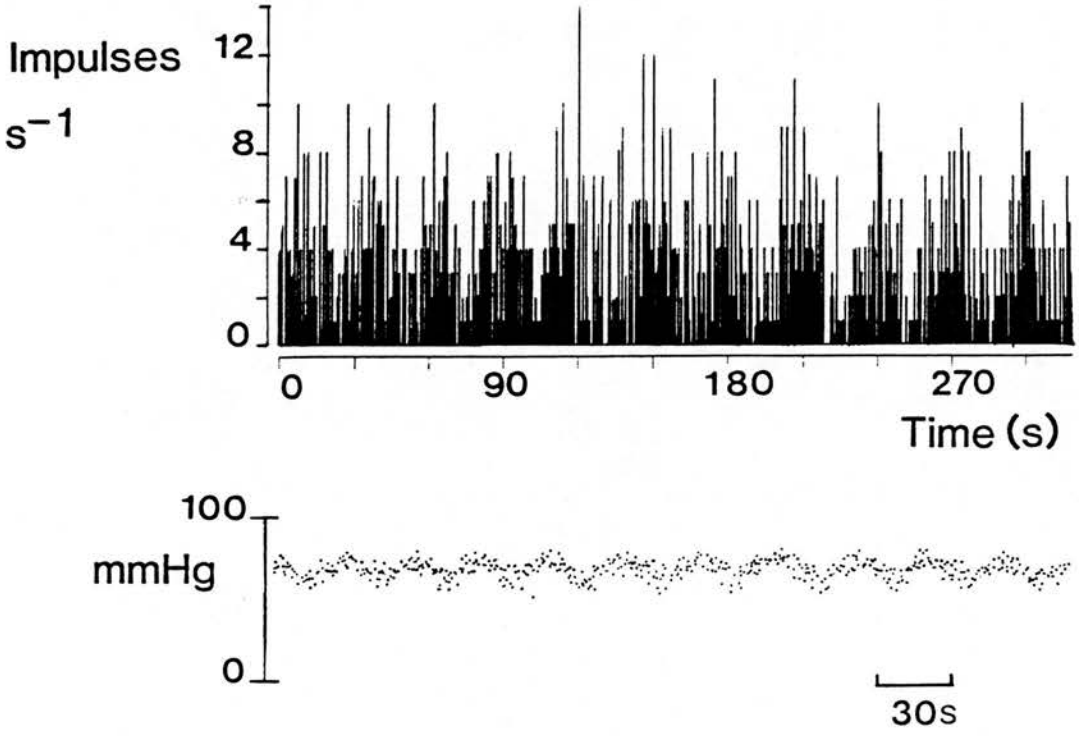

Impulses
s−1
12
8
4
0
0
90
180
270
Time (s)
100
mmHg
0
30s

Figure I7.

Injection of I00 ug of adrenaline B.P. in I0 ml of 9.0 % w.v. saline over 30 s (horizontal bar) into the left femoral vein caused :-

I. An increase in systemic blood pressure measured at the femoral artery (A) .

2. A decrease in abomasal body contractile activity (B) .

3. A decrease in abomasal antrum contractile activity and tone (C) .

Note that the effect on abomasal motility was longer-lasting than the effect on systemic blood pressure.

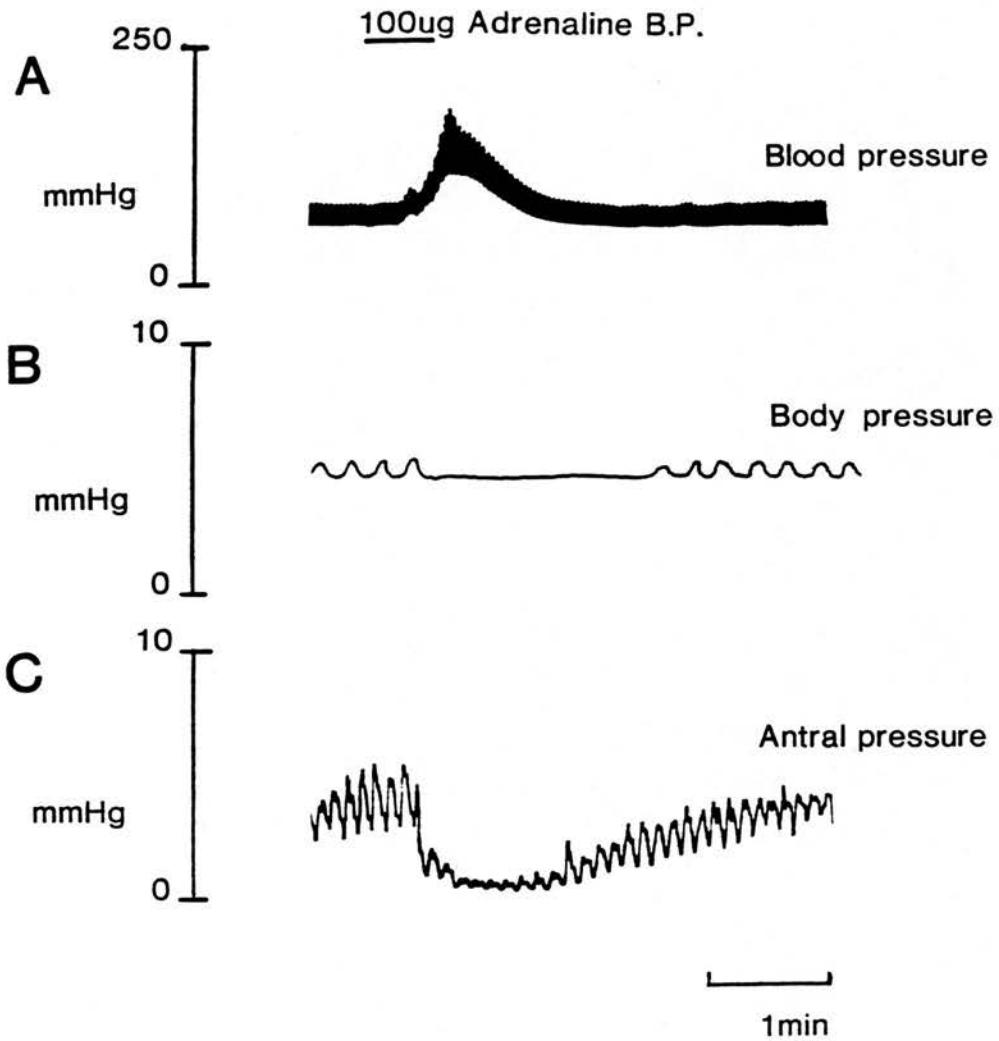

100ug Adrenaline B.P.
A
250
mmHg
0
Blood pressure
B
10
mmHg
0
Body pressure
C
10
mmHg
0
Antral pressure
1min

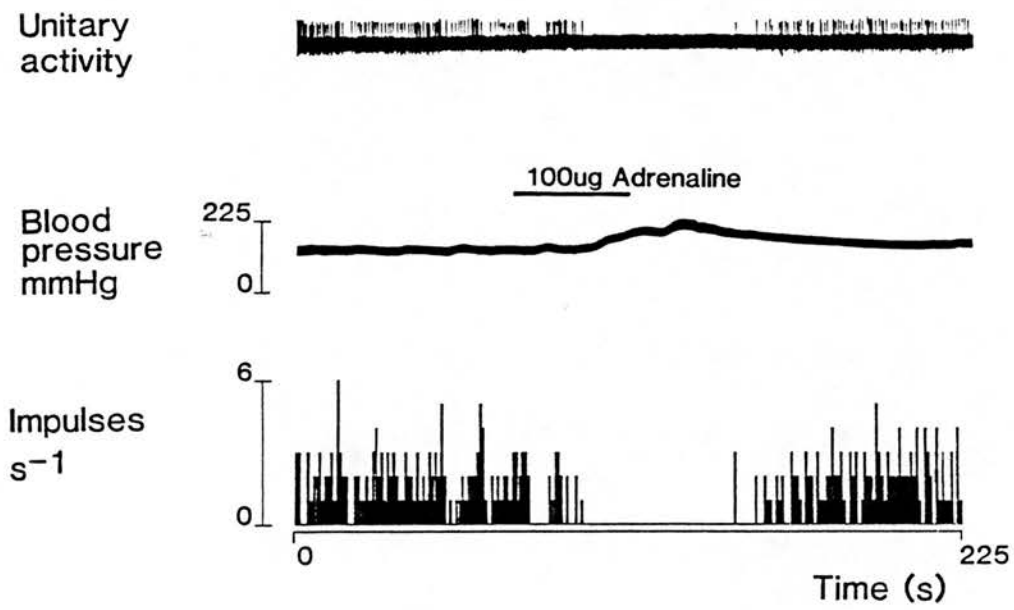
Figure 18.

Intravenous (femoral vein) injection of 100 ug of adrenaline in 10 ml of 9.0 % saline affected the discharge of single efferent units in the abdominal continuations of the dorsal and ventral vagi at the level of the abomasal antrum in one of 4 ways.

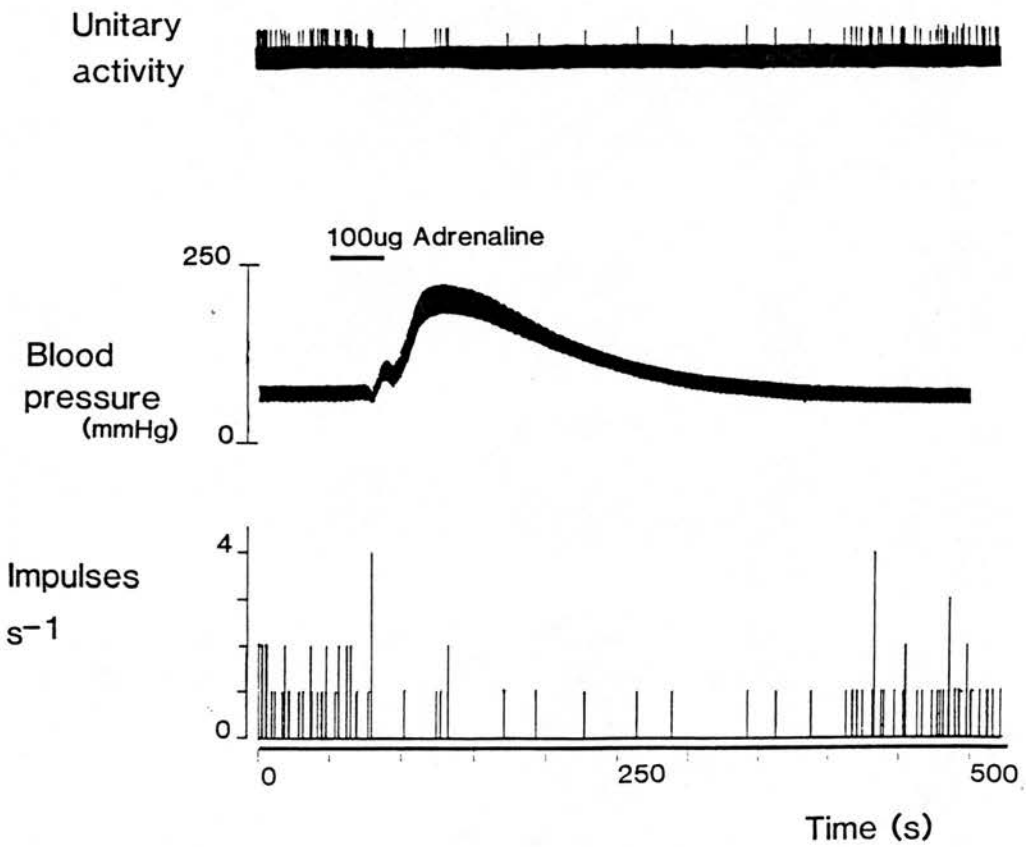
1. Abolition of discharge (A).
2. Reduction in discharge rate (B).
3. Increase in discharge rate (C).
4. No effect on discharge rate (D).

Each of the figures A, B, C and D shows the unit activity (upper trace), blood pressure (middle trace), and a frequency histogram of unitary activity (lower trace). The period of adrenaline injection is denoted by a horizontal bar in each case.

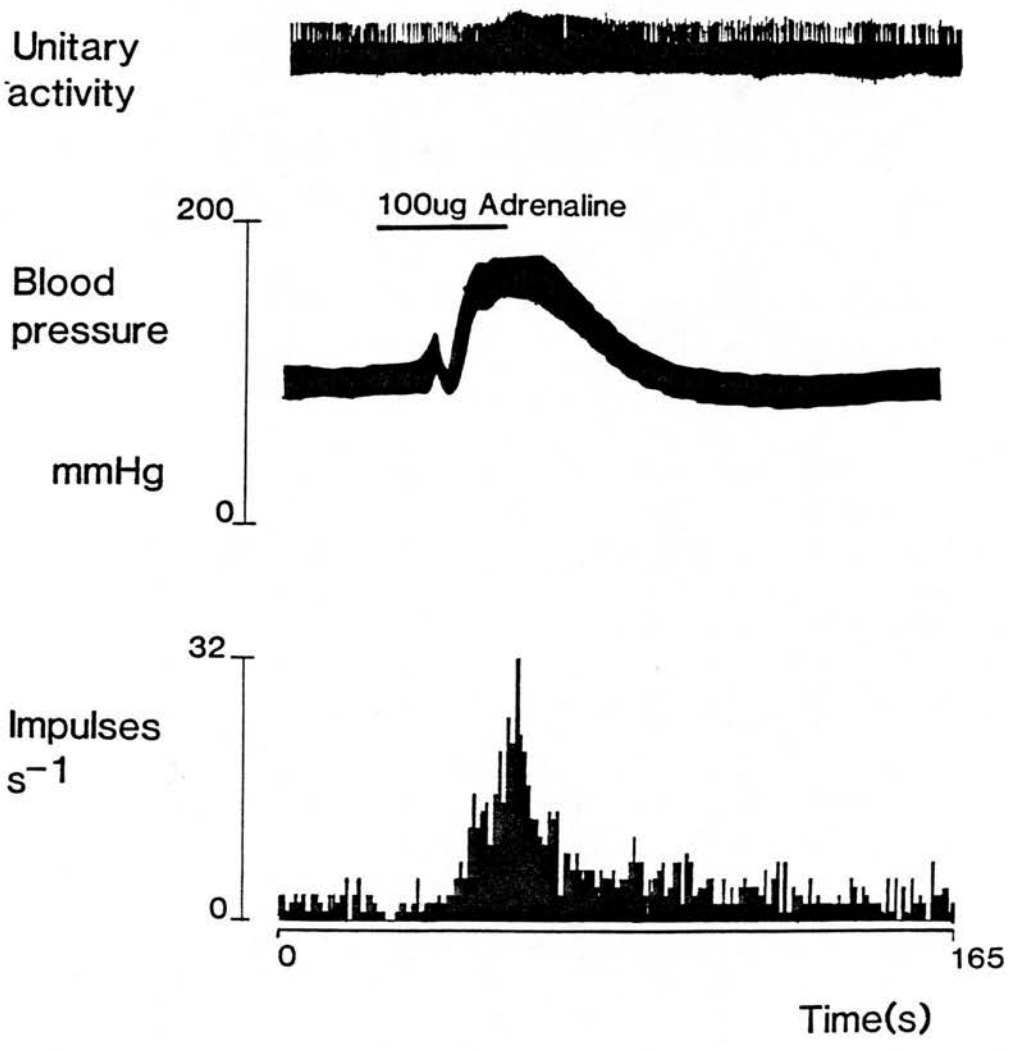
A


Unitary activity
100ug Adrenaline
Blood pressure mmHg
225
0
Impulses s−1
6
0
0
225
Time (s)

B


Unitary activity
100ug Adrenaline
250
Blood pressure (mmHg)
0
Impulses s−1
4
0
0
250
500
Time (s)

C


Unitary activity
200
100ug Adrenaline
Blood pressure
mmHg
0
32
Impulses s⁻1
0
0
165
Time(s)

D

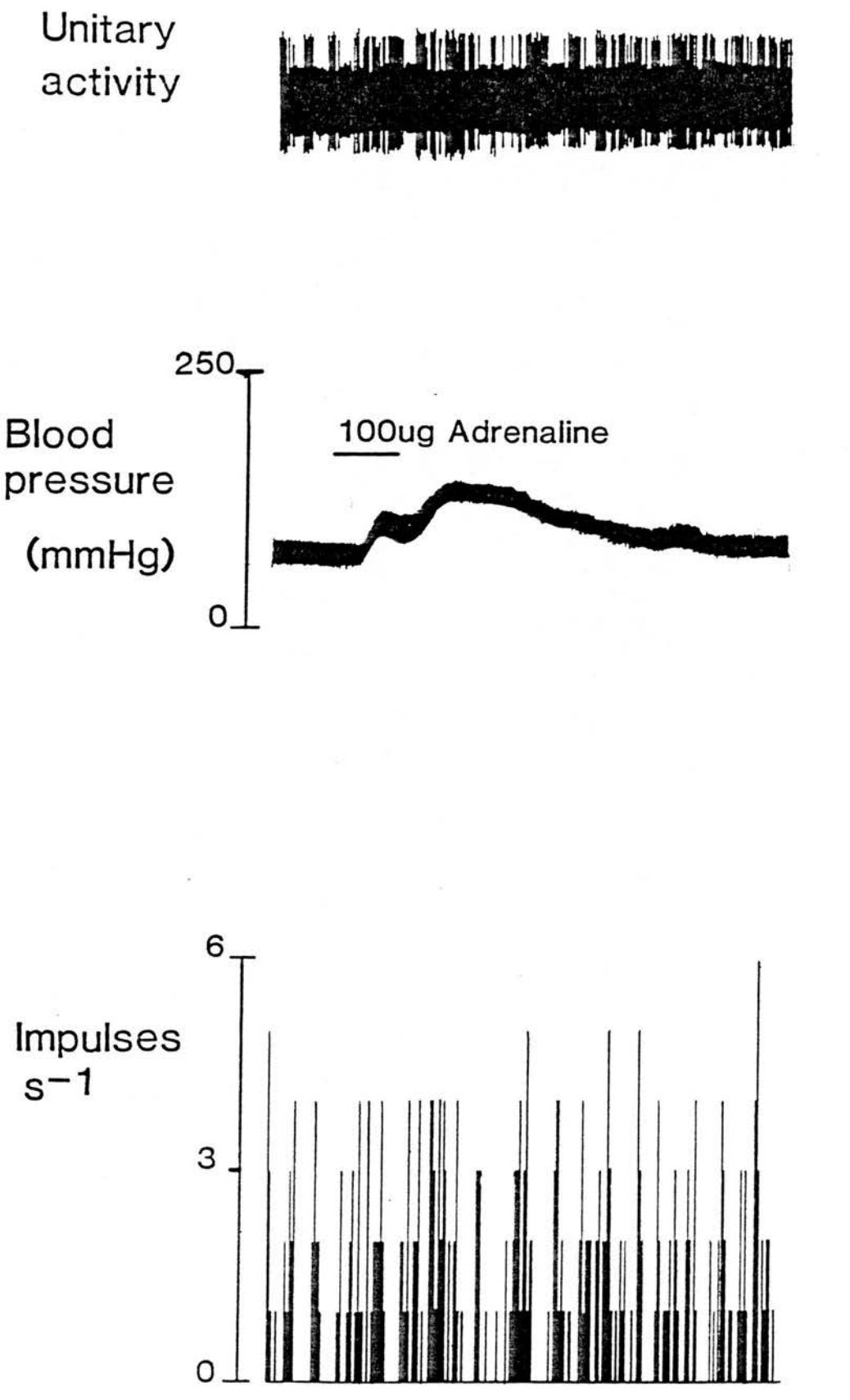

Unitary activity
250
Blood pressure
(mmHg)
0
100ug Adrenaline
6
Impulses s−1
3
0
0
250
Time (s)

Figure 19.

Intravenous adrenaline injection increased arterial pressure and decreased the discharge rate of 6 (18.2 %) efferent units sampled from the abdominal continuations of the dorsal and ventral vagi close (2 cm) from the abomasal antrum. A graph of percentage decrease in discharge rate against percentage increase in arterial pressure gave a correlation coefficient of $r = 0.77$.

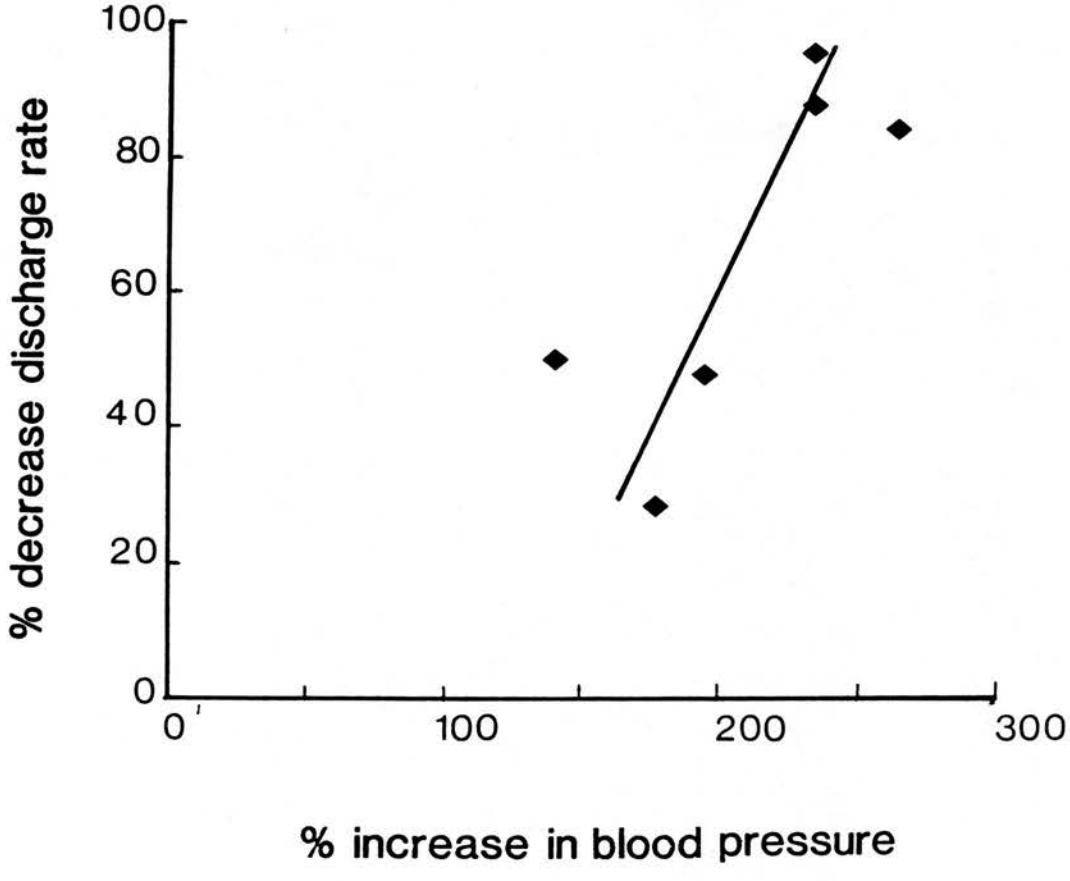

% decrease discharge rate
100
80
60
40
20
0
0
100
200
300
% increase in blood pressure

Figure 20.

Inflation of an abomasal body balloon with volumes of air between 10 and 250 ml abolished the discharge activity of 19.4 % of units sampled from the abdominal continuations of the dorsal and ventral vagi within 2 cm of the abomasal antrum. Discharge activity returned on deflation of the balloon. In this example the upper trace shows the spike train; the middle trace is a histogram of spike activity s^{-1}; the lower trace shows body balloon pressure.

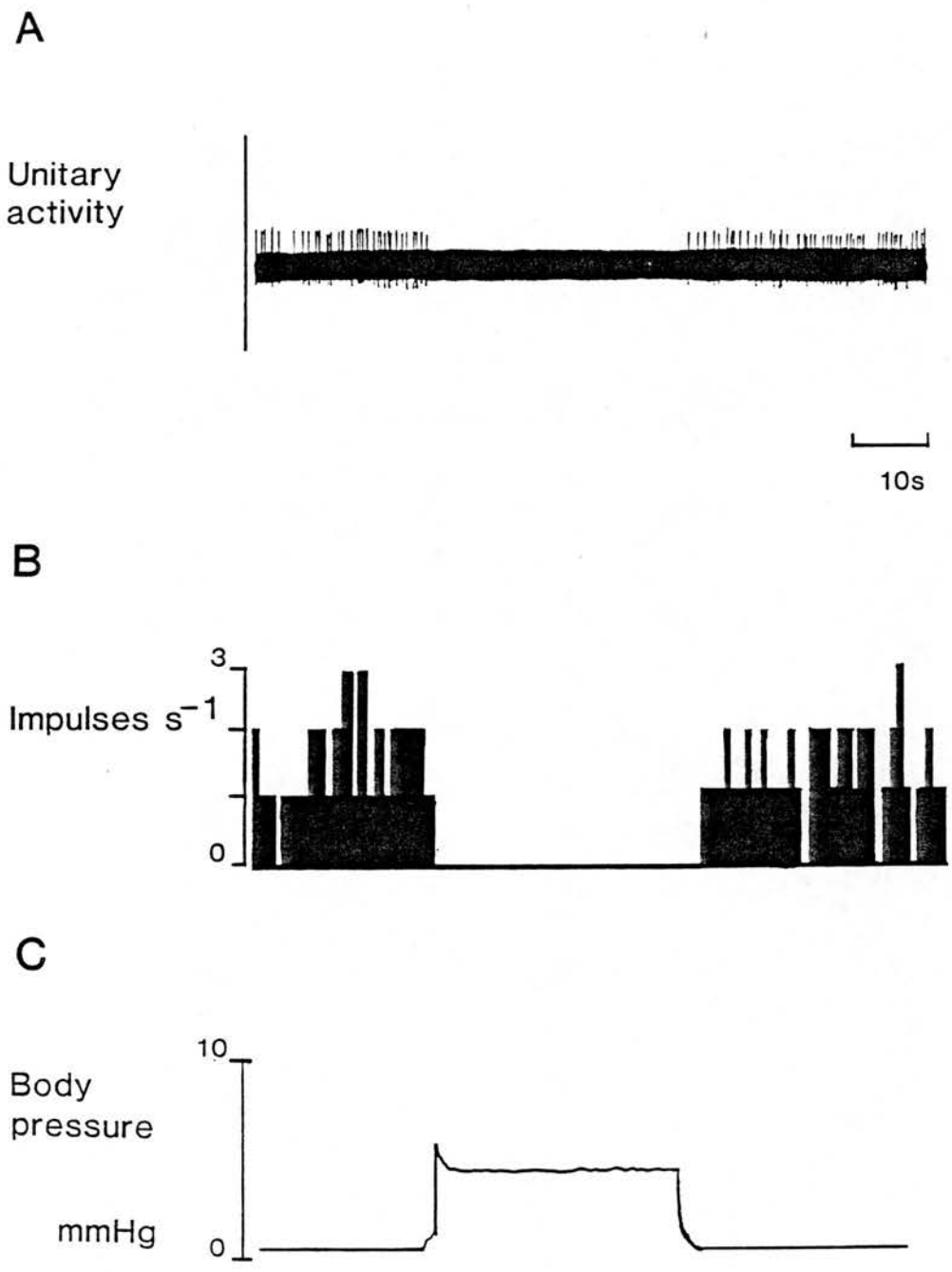

A
Unitary
activity
10s
B
3
Impulses s^{-1}
0
C
10
Body
pressure
mmHg
0

Figure 21.

A. Incremental (10-20 ml) inflation of an abomasal body balloon produced stepped reciprocal decrease in the discharge of 4 (12.9 %) units sampled from the abdominal continuations of the dorsal and ventral vagus within 2 cm of the abomasal antrum. Discharge activity returned on deflation of the balloon. The lower trace shows abomasal body pressure and volume; the upper trace shows the corresponding discharge activity s^{-1}.

B. A scattergraph (derived from the normalized results of all 4 units) of body pressure against residual discharge rate as a percentage of the spontaneous discharge rate. The correlation coefficient (r) was -0.85.

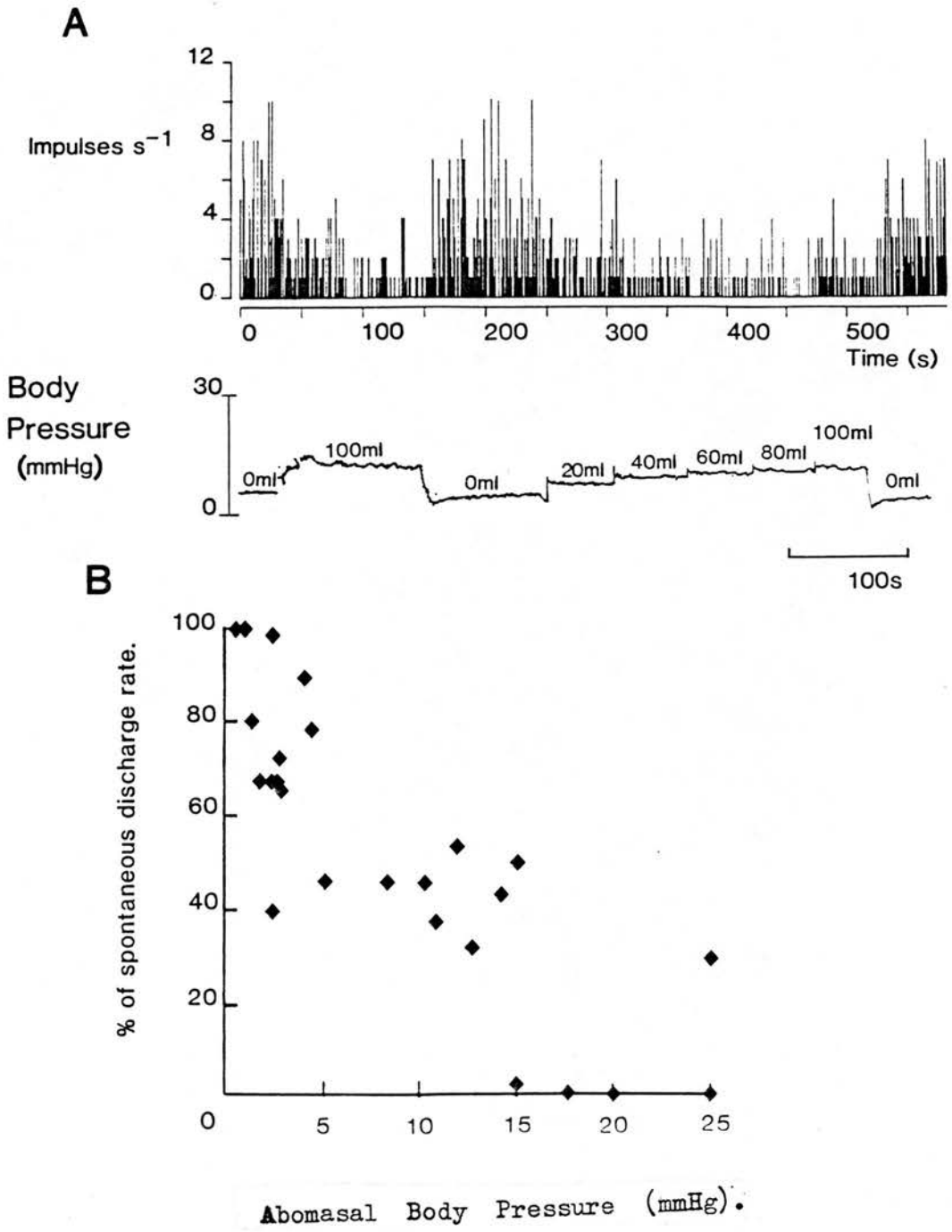

A
12
8
4
0
Impulses s^{-1}
0
100
200
300
400
500
Time (s)
Body
Pressure
(mmHg)
30
0
0ml
100ml
0ml
20ml
40ml
60ml
80ml
100ml
0ml
100s
B
100
80
60
40
20
0
% of spontaneous discharge rate.
5
10
15
20
25
Abomasal Body Pressure (mmHg).

Figure 22.

Post stimulus time histogram with respect to the R-wave f the e.c.g. of the discharge activity of a single unit issected from the abdominal continuation of the dorsal agus within 2 cm of the abomasal antrum. Bin width was 0 ms. The dashed horizontal line shows the mean discharge ctivity per bin. An estimation of the 'degree of cardiac hythmicity' was obtained by taking the difference between he maximum activity and minimum activity (each taken over bins) as a percentage of the mean. Maximum and minimum ctivity are marked by brackets. The 'degree of cardiac hythmicity' of this unit was 99.2 %.

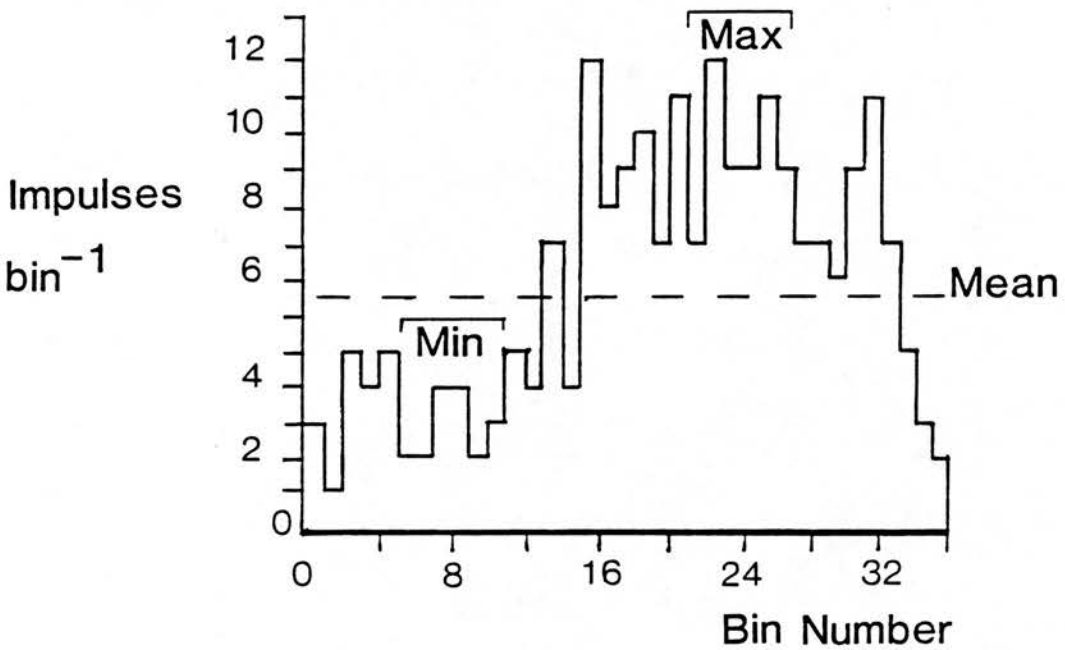
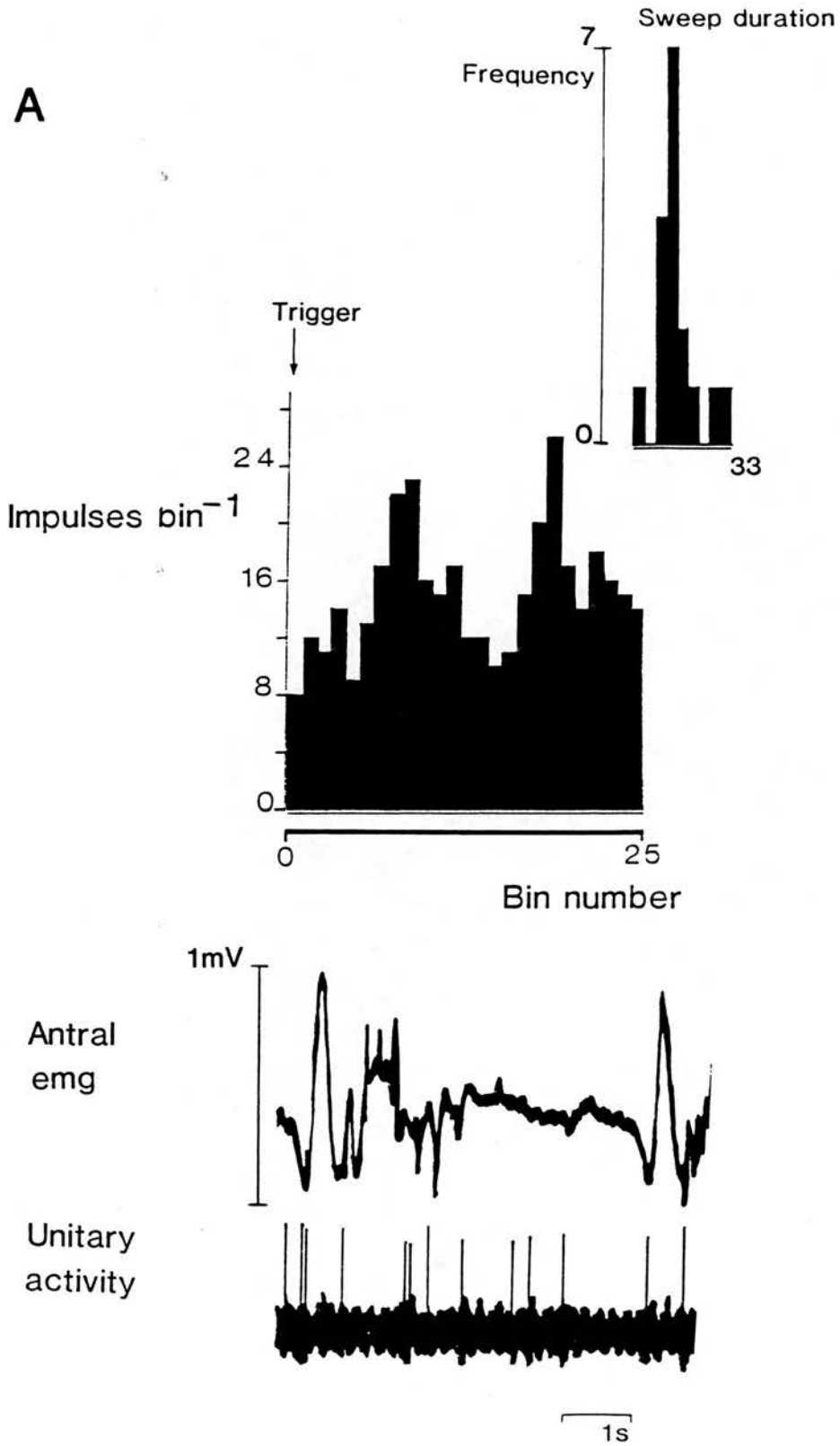

Max
12
10
Impulses
bin^{-1}
8
6
Mean
Min
4
2
0
0
8
16
24
32
Bin Number

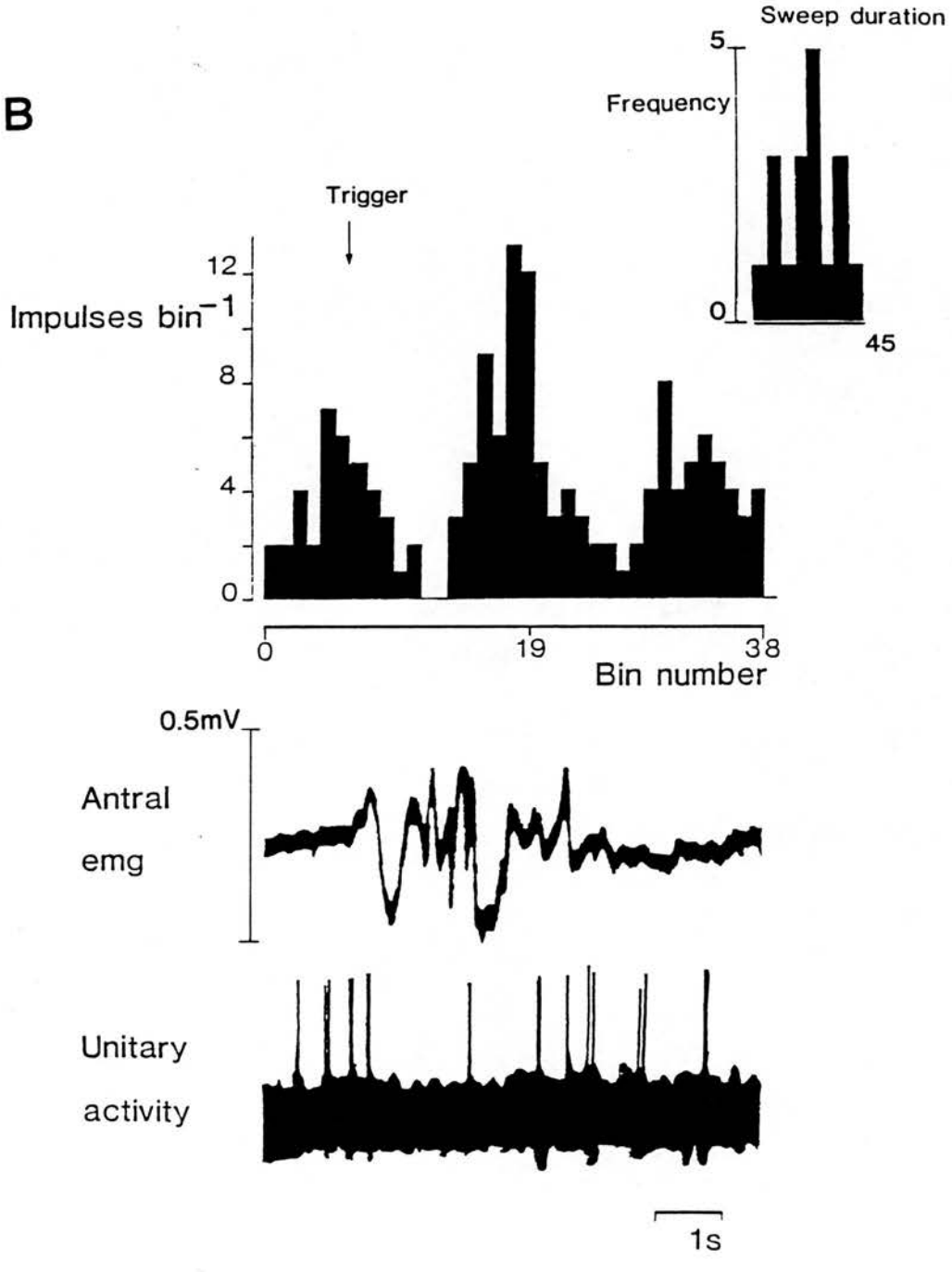
Figure 23.

Observation of post stimulus time histograms with respect to the start of antral e.m.g. electrical spiking activity (denoted by the arrow labelled 'trigger') of discharge activity recorded from efferent units dissected from the abdominal continuations of the dorsal and ventral vagi within 2 cm of the abomasal antrum revealed patterns of periodicity; a 2:1 ratio (A) and a 3:1 ratio (B). C is such a post stimulus time histogram of a unit with low antral rhythmicity and no obvious antral periodicity.

In each case the post stimulus time histogram (upper trace), a sample of the antral e.m.g. (middle trace), and a sample of the unitary discharge (lower trace) are given on the same time axis.

The histogram at the top right corner of each post stimulus time histogram gives the distribution of the duration of the intervals between the electrical spiking activity of the antral e.m.g. on a continuation of the time axis of the post stimulus time histogram. To ensure that all bins on the time axis in any one histogram were exposed to an equal number of sweeps the duration of the shortest electrical spiking activity interval was taken as the time axis for the post stimulus time histogram.


A
Sweep duration
7
Frequency
0
33
Trigger
24
Impulses bin^{-1}
16
8
0
0
25
Bin number
1mV
Antral
emg
Unitary
activity
1s


B
Sweep duration
5
Frequency
0
45
Trigger
12
Impulses bin⁻¹
8
4
0
0
19
38
Bin number
0.5mV
Antral
emg
Unitary
activity
1s

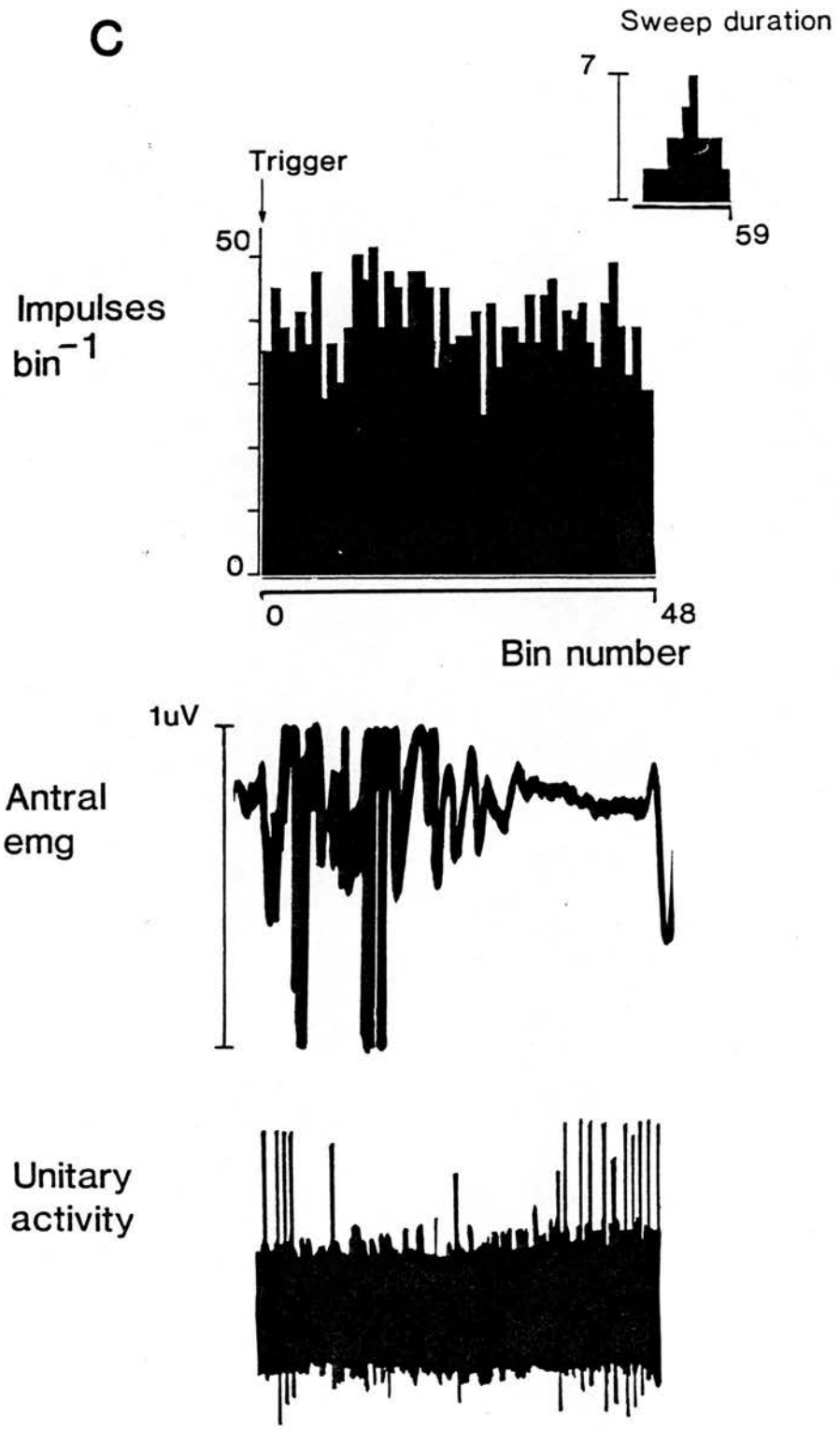
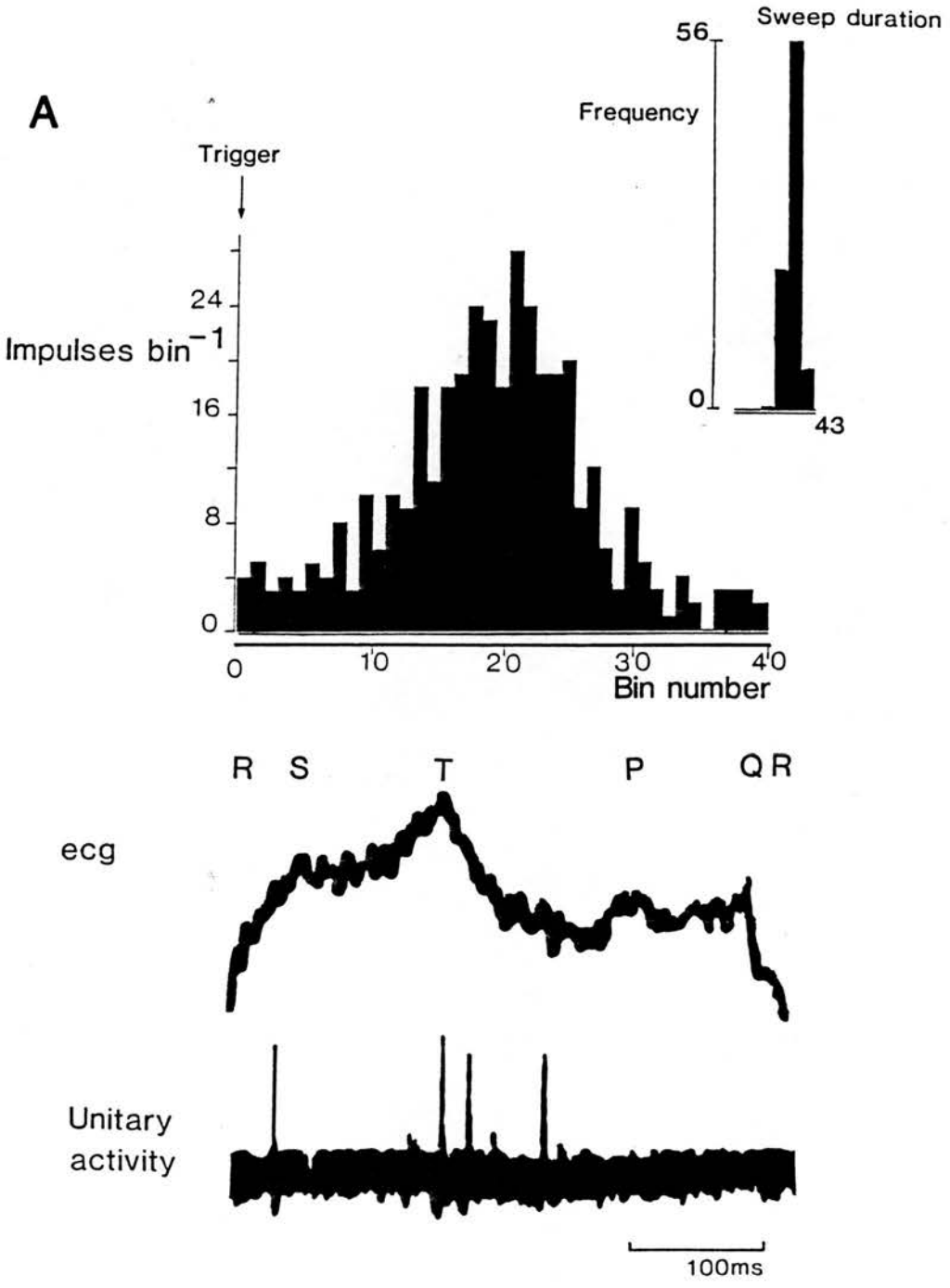

C
Sweep duration
7
59
Trigger
50
0
Impulses
bin^{-1}
0
48
Bin number
1uV
Antral
emg
Unitary
activity

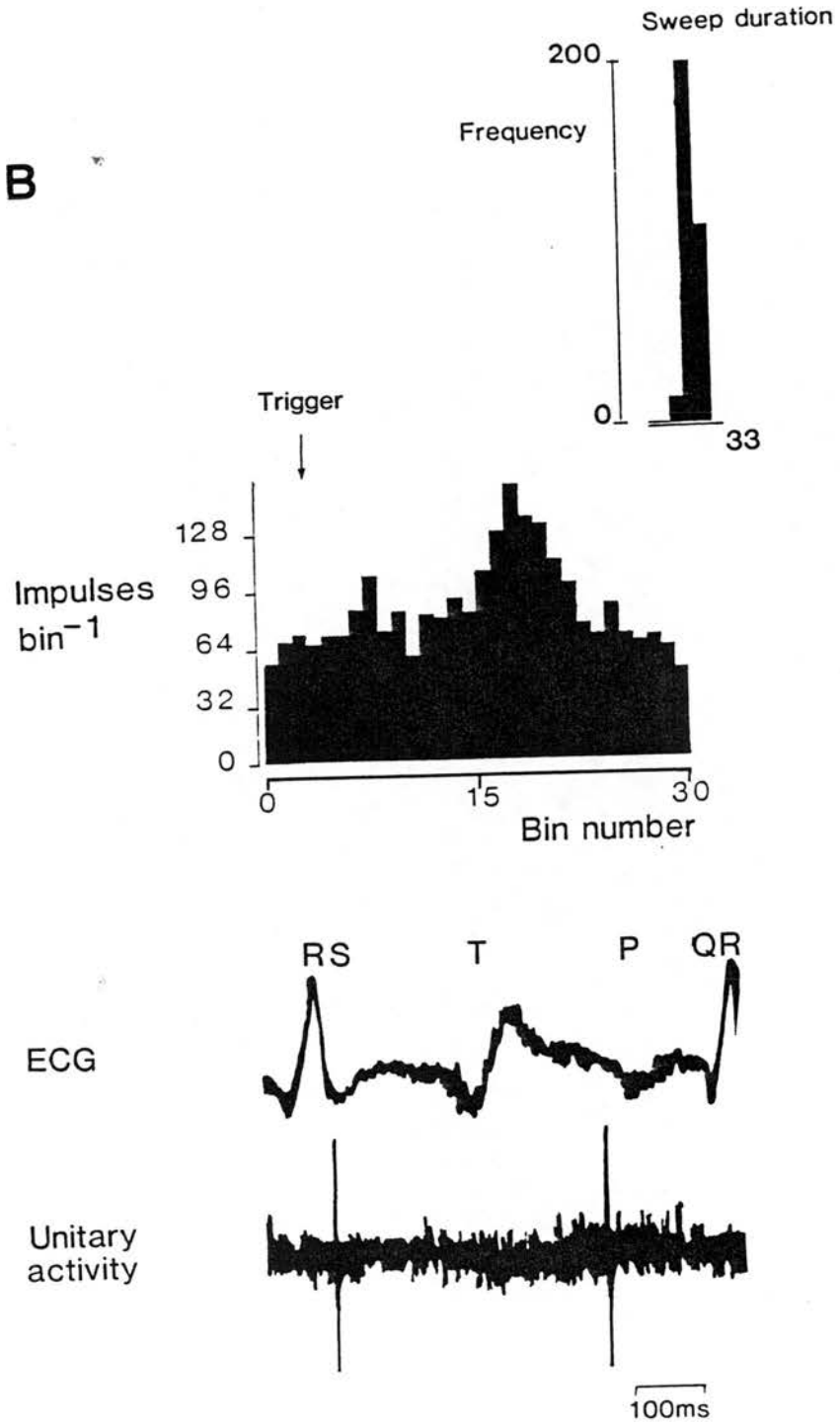
Figure 24.

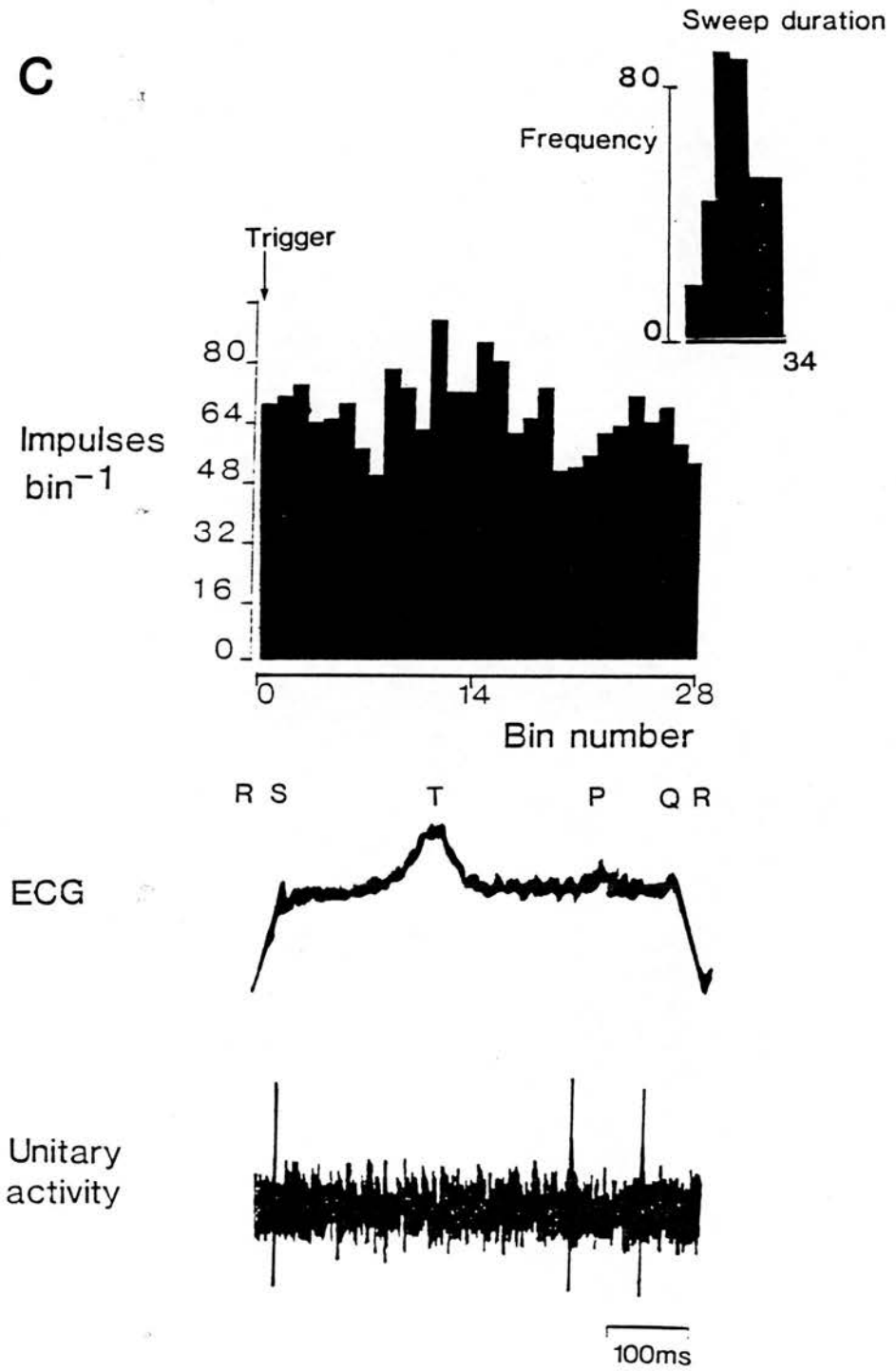
Observation of the patterns of post stimulus time histograms with respect to the R-wave (denoted by the arrow labelled 'trigger') of the e.c.g. of the discharge activity of efferent units dissected from the abdominal continuations of the dorsal and ventral vagi within 2 cm of the abomasal antrum revealed three periodicities of discharge: a 1:1 ratio (A), a 2:1 ratio (B) and a 3:1 ratio (C). D is such a post stimulus time histogram of a unit with a low degree of cardiac rhythmicity and no obvious cardiac periodicity. Bin width for the post stimulus time histograms displayed here is 20 ms.

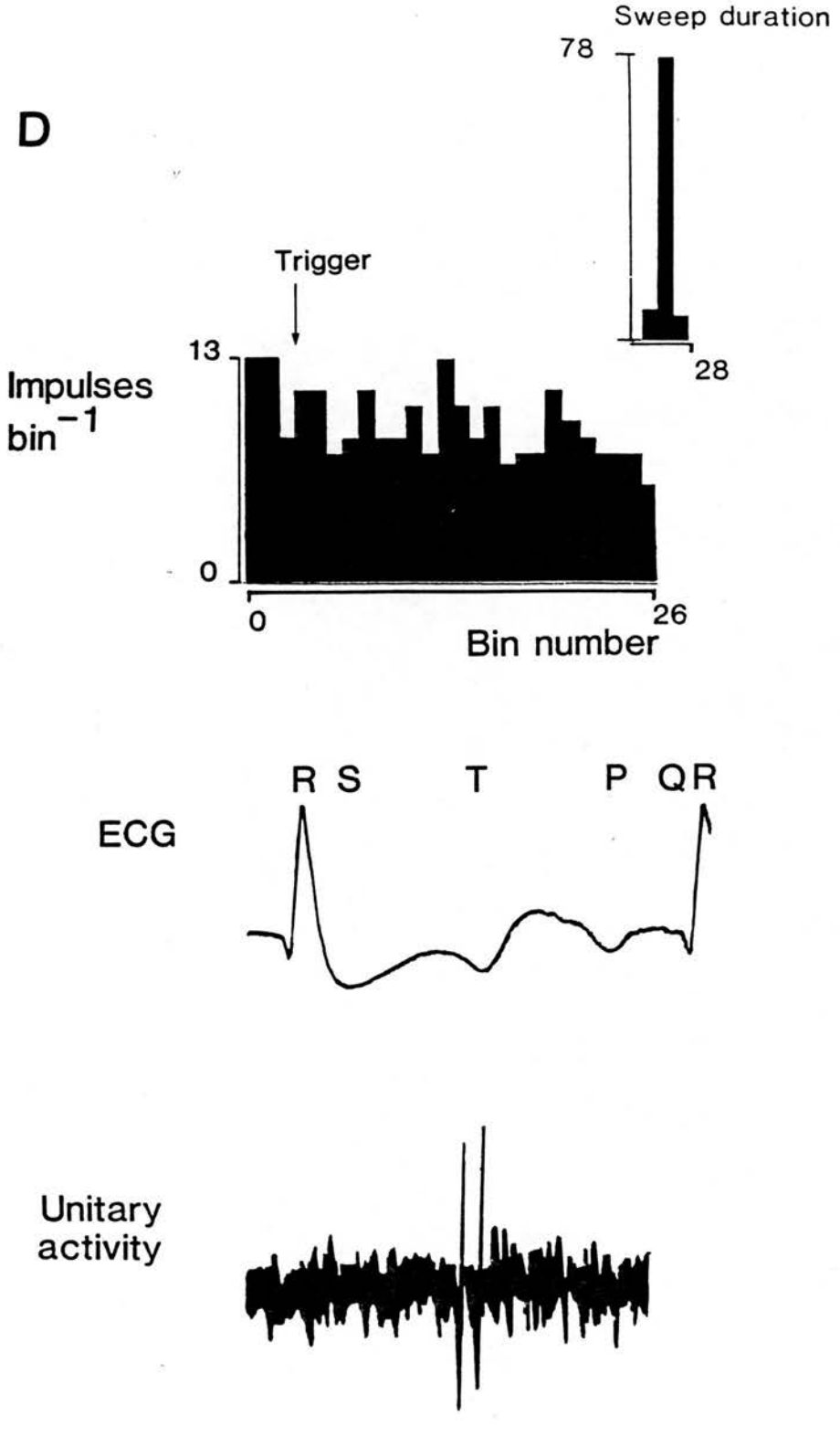
In each example the post stimulus time histogram (upper trace), a sample of the e.c.g. (middle trace) and a sample of the unitary discharge (lower trace) are given on the same time axis. The waves of the e.c.g. trace are labelled appropriately (P, Q, R, S & T).

The histogram labelled 'Sweep Duration' in the top right hand corner of each post stimulus time histogram gives the distribution of the duration of the inter-R-wave intervals on a continuation of the time axis of the post stimulus time histogram. To ensure that all bins on the time axis in any one histogram were exposed to equal numbers of sweeps the duration of the shortest inter-R-wave interval was taken as the time axis for the post stimulus time histogram.


A
Trigger
Sweep duration
56
Frequency
0
43
24
Impulses bin^{-1}
16
8
0
0
10
20
30
40
Bin number
R S T P Q R
ecg
Unitary activity
100ms


B
Sweep duration
200
Frequency
0
33
Trigger
128
96
64
32
0
Impulses
bin⁻¹
0
15
30
Bin number
RS
T
P
QR
ECG
Unitary
activity
100ms


C
Sweep duration
80
Frequency
0
34
Trigger
80
64
48
32
16
0
Impulses
bin⁻¹
0
14
28
Bin number
R S
T
P
Q R
ECG
Unitary
activity
100ms


D
Sweep duration
78
28
Trigger
13
0
Impulses bin^{-1}
0
26
Bin number
R S
T
P Q R
ECG
Unitary activity

CHAPTER EIGHT.

GENERAL DISCUSSION.

These experiments examined some aspects of neural mechanisms in abomasal motility in acutely prepared, chloralose-anaesthetized sheep. The application of electrophysiological and motility recording techniques to the transected abomasal preparation has enabled the aims of these experiments to be largely achieved. Abomaso-abomasal mechanisms involved in abomasal motility were identified; evidence was found to support the concept of a vagal inhibitory innervation of the adult abomasum; and the discharge activity of antral efferent units was characterized. The implications these findings have been discussed in the preceding text. However, it is important to consider the limitations of the techniques used, and to consider the place of the results as an entity in the general area of digestive physiology.

The function of the digestive system is to digest raw materials obtained from the environment into forms utilizable by the organism. Gastro-intestinal motility is one component of the more complex process of digestion; digestion is only one of the physiological processes of the organism. Thus the mechanisms controlling gastro-intestinal motility must cater for the ongoing digestive status in any part of the digestive tract, to accommodate the homeostatic demands of the organism, and adapt to cope with the demands placed on the organism by changes in the external enviroment. The formidible functional integration required

for this task is established by interaction between neural, muscular, exocrine, endocrine and paracrine factors. The primary aim of this study was to look at neural mechanisms regulating ovine abomasal motility. Several were identified. The functional significance of these neural mechanisms is difficult to gauge in anaesthetized animals without concurrent monitoring of the endocrine, exocrine or paracrine status of the animal, or without concurrent monitoring of the status of the rest of the alimentary tract. That abomasal motility was affected by factors outside those considered in these experiments was illustrated by the variability of the spontaneous motor profile of the abomasum in ostensibly identical preparations (Chapter 3). The neural mechanisms in abomasal motility described here are of relevance only when considered in the context of the abomasum as an integral part of a functional organ system co-ordinated by the complex interaction of a number of short-term and long-term regulatory influences.

The different and distinct spontaneous motor profiles of the abomasal body and antrum observed in these experiments (Chapter 3, figs. 2 & 3) support the concept of the body and antrum as two separate functional entities. This concept is further supported by the demonstrated differences in the neuronal mechanisms regulating their respective motor profiles. The body, having some contractile activity, may be functional in the mixing and transfer of abomasal contents. Bell and Grivel (1975) attributed propulsive function to the abomasal body of the

pre-ruminant calf. (Conversly, Grundy (1985) describes the muscle activity of the body/fundus of the monogastric stomach as 'limited to tonal changes'). However, the major function of the abomasal body may be regulatory; by influencing the motility of the forestomach and the antrum (Chapter 5, page 98) the body controls the rate of transfer of digesta into and out of the abomasum. The major function of the abomasal antrum is in aboral propulsion of digesta, although evidence has been found to suggest that the antrum may influence the body motor profile (Chapter 3, page 53). A functional distinction of the body/fundus and antrum of the monogastric stomach is also recognized. The monogastric body/fundus area allows receptive relaxation (Abrahamsson and Jansson, 1969), regulates antral motility (Andrews, Grundy and Scratcherd, 1980) and is involved in satiety (Janowitz and Grossman, 1949). The monogastric antrum is functional in digesta transfer (Hunt and Knox, 1969), regulating body tone (Abrahamsson, 1973a) and regulating its own activity (Deloof, and Rousseau, 1985). Fundamental differences were found between the neural mechanisms of control of the motor profile of the adult abomasum and the neural mechanisms of control of the motor profile of the monogastric stomach. Thus it is inappropriate to use the adult abomasum as a model for investigating monogastric stomach physiology. The assumption of Bell, Holbrooke and Titchen (1977) that the pre-ruminant abomasum is equivalent to the monogastric stomach in terms of factors regulating abomasal emptying has yet to be proved.

Evidence was found to support the concept that abomasal

motility is influenced both by intrinsic and extrinsic nerve supplies (Chapters 3, 4, 5 & 7). The interdependence of the extrinsic and intrinsic nerve supplies in regulating gastrointestinal motility is apparent. The extrinsic system modulates the activity of intrinsic efferents to provide the final link in the centrally-arising command chain. Duncan (1953) has shown that after vagotomy the intrinsic plexus in the forestomach of sheep does not provide sufficient gastrointestinal co-ordination to maintain life. Identification of intrinsic neural mechanisms regulating abomasal motility (Chapters 3, 4 & 5) supports the concept of the intrinsic plexus as an independent integrative system co-ordinating abomasal motility in conjunction with the C.N.S., rather than being merely a relay network for centrally originating commands. This concept is further supported by the persistance of tonic and contractile components of the abomasal motor profile in the absence of extrinsic innervation (Chapter 5, fig. 12).

It has been the practice in the past to consider that vagally-mediated excitatory effects are mediated by excitatory fibres and vagally-mediated inhibitory effects are mediated by inhibitory fibres. However, the results of Andrews, Grundy and Lawes (1980) in ferrets and Lisander and Delbro (1987) in cats suggest that vagal inhibitory fibres may have an indirect excitatory effect on gastric motility by showing that splanchnic inhibitory reflexes to the ferret stomach are normally suppressed by vagal activity. Evidence was found in the chloralose-anaesthetized sheep to suggest that the vago-vagal body-

antral excitatory reflex (Chapter 5, page 100) was achieved by a reduction in activity of antral efferents (Chapter 7, page 133). The interaction of excitatory and inhibitory fibres in gastrointestinal reflexes requires clarification.

Neural mechanisms affecting the amplitude of abomasal antral contraction were identified in these experiments (Chapter 3, page 35; Chapter 4, page 53 ; Chapter 5, page 99). In the monogastric animal antral contraction amplitude is subject to feedback inhibitory control from a diversity of enteroreceptors located at a number of sites. Distension of any portion of the gastrointestinal tract inhibits antral activity via the splanchnic nerves (Youmanns, 1968). Infusion of tryptophan, acid, fat and hypertonic solutions into various regions of the small intestine inhibits stomach emptying in the dog (Cooke, 1977). In rats cutaneous mechanical stimulation can inhibit antral motility via somatosplanchnic reflex arcs (Sato, Sato, Shimata and Torigata, 1975). No work of similar nature has been conducted in the adult sheep. In the conscious preruminant calf abomasal emptying is inhibited by duodenal infusion of acid, bicarbonate ions and hypertonic solutions (Bell and Razig, 1972; Bell, Nouri and Webber, 1980; Bell, Green, Wass and Webber, 1981). The function of the duodenal mechanoreceptors and chemoreceptors identified in the proximal duodenum of adult sheep (Cottrell and Iggo, 1984a, 1984b) has yet to be determined. Thus the experiments described here cover only one aspect of the neural control of abomasal motility. The

fact that anaesthesia appears to dissociate the co-ordination between antral and duodenal motility suggests that the influence of the duodenum on the abomasal motor profile is best investigated in conscious preparations.

The results obtained from the electrophysiological and discharge analysis techniques (Chapter 7) employed in these experiments have interesting implications for the study of visceral efferent function and destination:-

a). Recording visceral efferent activity close to the target organ allows assumptions to be made about efferent destination that cannot be made when recording at a site distant from the target organ. 'Close' recording avoids bias in the sampling of efferent units to be studied as the technique does not select between myelinated and unmyelinated fibres, vagal and splanchic fibres, or on the basis of discharge pattern. Thus, subject to anatomical constraints, it has advantages over the 'remote' recording techniques employed in gastric neurophysiology. This is not to say that recording at a site distant from the target organ does not have its place as a technique in efferent neurophysiology; an understanding of the neural mechanisms controlling reticuloruminal motility were achieved by recording motoneurone activity at the central level (Harding and Leek, 1971, 1972b). Thus whether a 'close' or 'remote' recording technique is appropriate for an experiment depends on the aims of the experiment and on the presence or absence of characteristic activity patterns in the target organ.

b). In these experiments units were classified according to their discharge response to application of rapid-onset, rapid-offset stimuli, and by P.S.T.H. analysis of discharge activity over a maximum period of 300 s. These techniques revealed little about the functional interaction of antral efferent activity and the antral motor profile. Simultaneous recording of antral efferent activity and antral muscle activity over a period of hours might reveal a relationship between unitary activity and the spontaneous changes observed in the antral motor profile. This would require recording e.m.g. activity close to the site of efferent destination; the lack of correlation often seen between antral e.m.g. and pressure recordings illustrates the difficulties in investigating microsopic functional relationships using macroscopic recording techniques.

c). The results of these experiments indicate that the discharge parameters by which visceral efferent activity is normally defined (namely discharge response to stimulation of enteroreceptor populations and the presence of system-related discharge patterns) may not be sufficiently discretionary to define visceral efferent function. The protocol for these experiments was based on the concept of efferent specificity; the results do not entirely support this concept. The presence of both cardiovascular and motility regulating efferent characteristics in the discharge of a unit is likely to reflect interaction of convergent afferents and central control circuits. The functional implication of this interaction may be that some antral efferents may regulate the activity of both vascular and

motility-related smooth muscle. This is upheld by the anatomy of efferent axons of the intrinsic plexi; neurotransmitter release occurs not from a terminal endplate, but from varicosities distributed along the length of the axon (Gabella, 1981).

FURTHER INVESTIGATIONS.

1. The possible existence of a functional barrier between the abomasal body and the abomasal antrum might be investigated by taking sequential pressure recordings along the length of the abomasum in order to identify a high pressure zone representing a functional sphincter between the body and antrum. This might best be done in a chronically-prepared standing animal so not to overlook the influence of gravity.

2. The presence of an inhibitory drive from the body to the antrum (Chapter 3, page 55) and a possible excitatory drive from the antrum to the body (Chapter 3, page 53) mediated through the intrinsic plexi has been demonstrated. Neural correlates to these influences might best be investigated by combined microelectrode and neuropharmacological techniques in an in vitro abomasal preparation.

3. Electrical stimulation of the cut peripheral end of the cervical vagus provided evidence of a vagal inhibitory innervation to the body (Chapter 4, page 69). Further evidence might be obtained by observing the effects on the abomasal body of electrical stimulation of the deafferented vagus. A functional correlate for the proposed vagal inhibitory innervation of the body has yet to be identified.

4. The abomasal body was demonstrated to have a limited ability to accommodate increases in volume without increases in pressure by a mechanism which was independent

of extrinsic innervation (Chapter 5, page 102). The intrinsic mechanisms eliciting this response have yet to be determined. It would be interesting to see if abomasal body smooth muscle shared the properties of low resting membrane potential and inherent elasticity displayed by their counterparts in the body/fundus of the monogastric stomach (Szursweski, 1981).

5. Electrical stimulation of the cut peripheral end of the cervical vagus produced antral inhibition in 3 preparations and antral excitation in 4 preparations (Chapter 4, page 53). Whether this is an artefact produced by a non-physiological stimulus or a demonstration of the integrative properties of the intrinsic plexi has yet to be determined.

6. These experiments did not examine the influence of the distal digestive tract on abomasal motility. The functional influence of duodenal sensory receptors on the abomasal motor profile of the adult sheep might best be determined in a conscious animal using a re-entrant cannula technique as described by Bell and Watson (1975) in the preruminant calf.

7. The discharge of a proportion of efferent units supplying the antrum responded to limited inflation of the body pouch (Chapter 7, page 133). The effect of larger volumes of body inflation on efferent discharge would require a preparation of greater stability. An alternative method would be to observe the response of antral efferent units to evoked body contraction by, for example,

electrical stimulation of the body wall.

8. The discharge of efferents supplying the abomasal antrum was demonstrated to have rhythms associated with the periodicity of the antral e.m.g. (Chapter 7, page 135). The origin of these rhythms (central or peripheral) might be determined by selective antral deafferentation and by identification of centrally-located independent oscillation circuits with periodicities related to antral motility.

9. A combination of electrophysiological and histological tracing techniques may be required to solve the problem of determining efferent destination and function. Current technology may be inadequate. The feasibility of such investigations depends on the validity of the concept of visceral efferent specificity. Establishment of this concept may be a prerequisite to further investigation of efferent function and destination.

IO. Intra-venous adrenaline injection altered the discharge rate of 27 out of 33 units tested. Whether the adrenaline induced change in discharge rate is related to changes in blood pressure could be elucidated in the following manner:-

a. Injection of adrenaline into the left gastric artery would differentiate between the abomasal and cardiovascular effects of systemic adrenaline injection.

b. Increasing blood pressure by non-pharmacological means such as occlusion of the carotid arteries or oxygen depletion.

c. Increasing blood pressure with more powerful and more specific pressor agents such as noradrenaline.

II. The validity of the recorded differences in body and antral tone could be checked using properly calibrated balloon catheters of equal compliance.

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